The Respiratory Physiology of the New Zealand Paddle Crab, *Ovalipes catharus*

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General Summary of Chapters 2 - 4

Ovalipes catharus is a back-burrowing brachyuran which makes temporary burrows in soft sandy sediments. Like many burrowing crabs, *O. catharus* shows prolonged periods of sustained reverse ventilation. Well settled individuals were found to exclusively reverse-ventilate, regardless of burial state. However following activity, both buried and unburied animals showed periods of forward ventilation. Immediately following 15 minutes of exercise by swimming, crabs remaining unburied spent an average of 75.4 ± 14.2% of the time forward ventilating, while crabs that were allowed to burrow in sand spent only 32.5 ± 12.1% of the time in the forward mode.

Previous studies suggest that buried crabs reverse the direction of ventilation in order to maintain a respiratory water stream by utilising superincumbent water. (Garstang, 1897b; Hartnoll, 1972; Taylor, 1984; McLay and Osborne, 1985; Otwell and Webb, 1990). Caine (1974) went further to suggest that interstitial oxygen tensions may be too low to sustain the oxygen demands of buried crabs. In laboratory studies the interstitial water in sediments inhabited by *O. catharus*, indeed, were found to be hypoxic. Values of interstitial Po_2 were as low as 50 % of that of the superincumbent water. Despite this, the present study has shown that both forward and reverse ventilation are utilised by *O. catharus*, regardless of burial state. When buried, P_io_2 is maintained at near normoxic levels during forward ventilation by the utilisation of superincumbent water for ventilation. This is enabled by the formation and use of exostegal channels which provide a conduit for water flow from above the sediment to the primary inhalent openings, the Milne-Edwards apertures.

In unburied *O. catharus*, branchial water flow patterns were found to be as described for other species (Hughes et al., 1969; McDonald et al., 1977). Water is drawn into the ventral hypobranchial space of the branchial chambers at the bases of the limbs and flows dorsally through the gills to the epibranchial space before passing anteriorly and being exhaled via ducts near the mouthparts. This flow is roughly countercurrent to the direction of haemolymph flow within the gills. When the direction of ventilation was reversed, this pattern of flow was disrupted. Anteriorly, water enters the prebranchial chambers, where the scaphognathites are situated before passing into the epibranchial space. Much of this water flows posteriorly and is exhaled via apertures between the fourth and fifth pereiopods. Some exhalent flow appears at the Milne-Edwards apertures suggesting some concurrent exchange may be possible. From the major sites of inhalation, the patterns of flow appear to be similar in buried crabs. However, the proportions of flow exiting each aperture were highly

variable, suggesting an ability to regulate patterns of gill irrigation in this species.

In unburied crabs, the mean oxygen extraction efficiency was significantly lower and the mean convection requirement ($\dot{V}_w/\dot{M}o_2$) was significantly higher during periods of reverse ventilation ($E_w\%$ = 23.7 ± 1.8%; $\dot{V}_w/\dot{M}o_2$ = 18.4 ± 1.7 ml μmol^{-1}) when compared to adjacent periods of forward ventilation ($E_w\%$ = 32.9 ± 4.1%; $\dot{V}_w/\dot{M}o_2$ = 13.9 ± 1.9 ml μmol^{-1}). In contrast, in buried crabs, values of $E_w\%$ and $\dot{V}_w/\dot{M}o_2$ were similar during adjacent periods of forward ($E_w\%$ = 53.3 ± 4.6%; $\dot{V}_w/\dot{M}o_2$ = 10.5 ± 1.4 ml μmol^{-1}) and reverse ventilation ($E_w\%$ = 49.4 ± 2.9%; $\dot{V}_w/\dot{M}o_2$ = 9.7 ± 0.5 ml μmol^{-1}). The lower values of $E_w\%$ and higher values of $\dot{V}_w/\dot{M}o_2$ recorded from unburied reverse ventilating crabs are presumably due to disruption of the counter-current gill perfusion/irrigation relationship that exists when ventilating in forward mode. When buried, the improved efficiency of oxygen extraction and convection requirements seen during periods of reverse ventilation compared to unburied crabs utilising this mode, may be due to better irrigation of the gills resulting from regulation of the branchial water flow pathways, or to utilisation of an alternative site for gas exchange. The potential of the branchiostegal lining of the branchial chambers to fulfil such a role was examined. From vascular corrosion casts it was apparent that this region is well supplied with venous haemolymph which returns to the pericardium without being reoxygenated at the gills. This would create a large Po_2 gradient across the cuticle of the branchiostegite which is relatively thin (10 - 20 μm), thus diffusion of oxygen across this barrier and into the haemolymph may be possible. Possible mechanisms of regulation of perfusion of the gills and branchiostegites are discussed.

In unburied crabs, branchial chamber pressure (P_{branch}) was similar in magnitude, but of opposite sign, in the two ventilatory modes. In buried animals, values of P_{branch} were much greater than those recorded from unburied crabs, and P_{branch} was significantly greater during periods of forward ventilation than reverse ventilation. The increased values of P_{branch} in buried crabs indicate a greater resistance to ventilatory water flow, especially when ventilating in the forward direction. This increase in ventilatory resistance affected scaphognathite function in two main ways: Firstly, mean scaphognathite stroke volume ($\dot{V}_s$) in buried crabs was reduced compared to unburied animals. In forward and reverse ventilating unburied animals, mean V_s was similar at 4.86 ± 0.14 ml beat^{-1}kg^{-1} and 4.92 ± 0.15 ml beat^{-1}kg^{-1}, respectively, while in buried crabs, mean values of $\dot{V}_s$ were 3.63 ± 0.14 ml beat^{-1}kg^{-1} and 4.11 ± 0.14 ml beat^{-1}kg^{-1} in forward and reverse ventilation, respectively. Secondly, as a result of the higher values of P_{branch} recorded from buried animals, ventilatory stroke work (W_s) and ventilatory power (W_r) were increased when buried, especially when utilising the forward mode.

By converting ventilatory power terms to oxygen equivalents and using efficiency values from the literature (Wilkens et al., 1984), estimates of the energetic

cost of ventilation (ie. the fraction of total $\dot{M}o_2$ that is devoted to ventilation) were calculated for the four treatment groups at a given ventilatory flow ($\dot{V}_w$ = 0.6 l kg^{-1} min^{-1}). In unburied animals, the ventilatory power and oxygen requirements were similar in the two modes. However, as a result of the lower values of E_w% and $\dot{V}_w/\dot{M}o_2$ recorded during reverse ventilation, $\dot{M}o_2$ was lower at a given $\dot{V}_w$ in this mode. Because of this, a higher proportion of total $\dot{M}o_2$ (23.6%) was required for ventilation in the reverse mode, than the forward mode (12.1%).

In buried crabs, E_w%, $\dot{V}_w/\dot{M}o_2$ and $\dot{M}o_2$ were all similar at a given $\dot{V}_w$. However, the increased ventilatory resistance seen in the forward mode, required an increased power output from the ventilatory muscles when generating flow, compared to that during reverse ventilation. This translates into an increased oxygen requirement of the ventilatory musculature. As a result, the estimated ventilatory fraction during forward ventilation was higher (43.3%) than during reverse ventilation (20.0%).

The predominant modes of ventilation in buried and unburied crabs following exercise reflect these differences. Unburied crabs primarily utilise forward ventilation while buried crabs utilise reverse ventilation. It is suggested that *O. catharus* modify their ventilatory behaviour depending on environmental factors and the internal physiological state of the animal, in order to reduce the overall energetic cost of ventilation. Potential mechanisms of control of ventilatory switching are discussed.

A summary of Chapter 5 (*The Physiological Effects of Commercial Processes*) can be found on page 102.

Chapter 1

General Introduction

The New Zealand paddle crab, *Ovalipes catharus* is a swimming crab belonging to the family Portunidae (Fig. 1.1). *O. catharus* is a subtidal species distributed throughout New Zealand along sandy beaches and in estuaries to depths of about 10 m. However, typical of many portunid species, *O. catharus* is a capable swimmer and migrates large distances with large swarms of crabs being reported in surface waters several kilometers offshore. The maximum size attained by this species is approximately 150 mm carapace width in the males, corresponding to a weight of between 600 - 700 g. The maximum size attained by females is somewhat smaller.

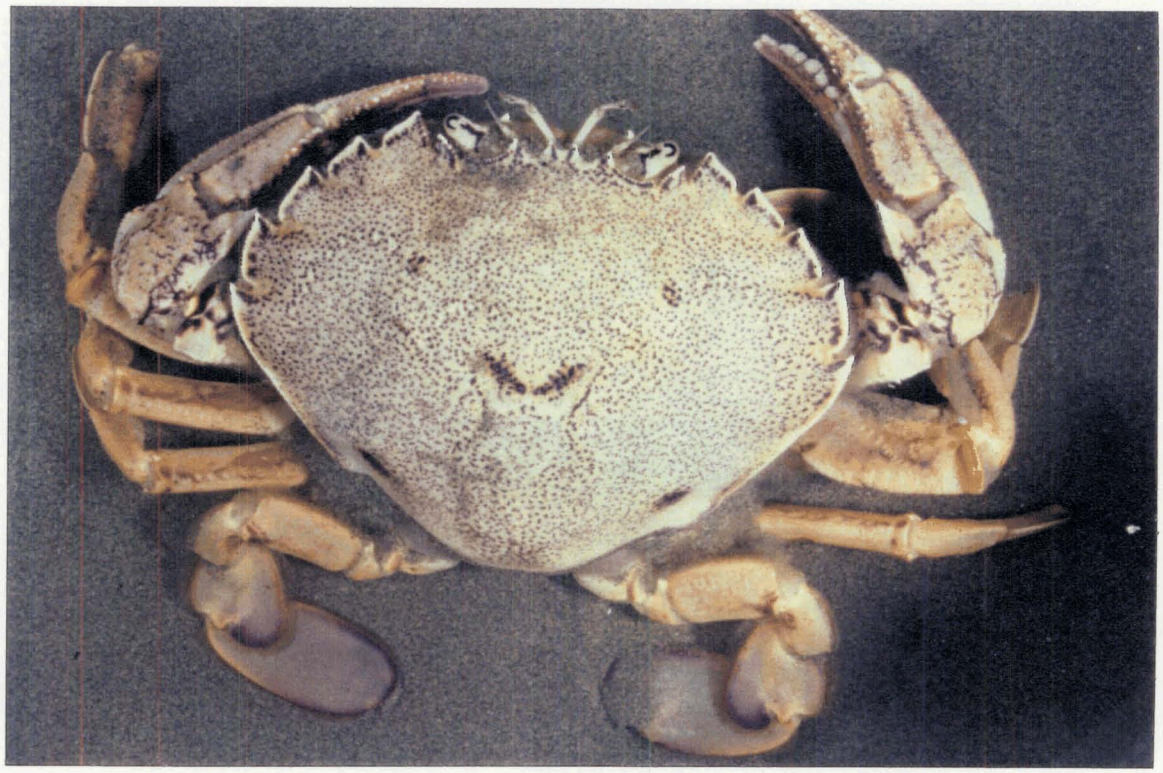

Fig. 1.1. A large male specimen of *Ovalipes catharus*. This animal is approximately 115 mm carapace width and weighs about 350 g. Note the flattened dactyls of the last pair of pereipods which are used for swimming and burrowing in sand. The scale bar = 1 cm.

During the daylight hours, individuals are generally found buried in sandy substrates. Using the flattened dactyls on the last pair of pereiopods (Fig 1.1), these crabs are able to rapidly bury themselves, taking an average time of about 6.5 seconds (McLay and Osborne, 1985). Once buried, only the antennae and eyes remain visible (Fig. 1.2). Individuals will remain buried for extended periods, leaving the substrate only for specific purposes, such as predator avoidance, migration, or for the capture of prey. If disturbed, a buried crab will quickly emerge from the sediment and either stand and fight, using the powerful chelae, or escape by swimming rapidly away at speeds of up to 1 m s^{-1}. Swimming is also achieved by using the paddle bearing legs as described for *Callinectes sapidus* (Spirito, 1972).

Fig. 1.2. A fully buried individual of *O. catharus* with only the eyes and antennae visible above the sediment surface.

This study is divided into two distinct sections. The first deals with the respiratory physiology of *O. catharus* in relation to it's natural habit. Specifically, the consequences of burial in sand, and reversed ventilation, for the functioning of the respiratory system. The second section is devoted to investigating the physiological effects of commercial processes used in the fishery for *O. catharus*, with the ultimate intention of assessing this species suitability for live aerial exportation to overseas markets.

Burial and Reverse Ventilation

In decapod crustaceans, the gills are contained within chambers on each side of the body, formed by outgrowths of the body wall. Primitively, there are four gills on each side of every thoracic segment and these arise on, or near the bases of the thoracic appendages. This would give a total of 32 gills on each side, but there are no known species with this number of gills. In *O. catharus* however, this number has been reduced to 9 gills on each side. The brachyura possess phyllobranchiate gills consisting of layers of thin plate-like lamellae. The gills are irrigated by water currents generated by the action of paddle-like projections of the second maxillae, the scaphognathites. During the course of normal forward ventilation, water is drawn into the branchial chambers through apertures at the bases of the chelipeds and walking legs. Typically, the large Milne-Edwards apertures encircling the bases of the chelipeds are the primary inhalent openings. Inhalent water is directed into the hypobranchial space between the gills and the body wall, before passing through the gill sieve to the dorsal epibranchial space and being exhaled anteriorly, via pores exiting near the mouthparts. Many decapod species also show periodic reversals of the ventilatory water current. These are produced by reversing the direction of beating of the scaphognathites. Most brachyuran, macruran, and anomuran species show brief reversals, lasting no more than 2 or 3 beats of the scaphognathites. The function of these events is unclear and may vary between species, however brief reversals have been likened to coughs and, in some species, appear to have a gill cleaning function (Arudpragasam and Naylor, 1966). Several burrowing brachyuran species, including *Ovalipes catharus*, have been shown to ventilate in the reverse direction for prolonged periods, especially when buried, and in some situations reverse ventilation has become the predominant mode (Garstang, 1987a, 1897b; Hartnoll, 1972; Caine, 1974; Taylor, 1984, McLay and Osborne, 1985). Reversing the direction of ventilation is thought to enable buried animals to maintain a relatively clean and

well oxygenated ventilatory water current, as the mouthparts and apertures of the prebranchial chambers, which become the inhalent openings during reverse ventilation, are usually close to the sediment surface.

Few observations have been made on the pattern of branchial water flow during reverse ventilation. The inhalent water must enter at the prebranchial pumping chambers where the scaphognathites are situated, via the prebranchial apertures on either side of the mouthparts. The water then presumably passes into the epibranchial space of the branchial chambers. Early studies by Garstang (1897a & b) showed that the exhalent water streams in two species of burrowing, reverse-ventilating crabs (*Corystes cassivelaunus* and *Portumnus nasutus*) appeared out of the apertures at the bases of the limbs. Precisely what route the water stream took within the branchial chambers was not clear and no subsequent studies have been undertaken to clarify this.

Ventilation in aquatic animals is typically an energetically expensive activity. Water is 800 times denser than air and contains 30 times less oxygen per unit volume than air. Thus the work involved in ventilating such a viscous medium is high, and the amount of oxygen available to be extracted by an animal is low. Wilkens et al. (1984) suggest that the work efficiency of the scaphognathite pump is as low as 3.15% in forward ventilating *Carcinus maenas*. As a result, the metabolic cost of ventilation is very high (30% of total metabolism at rest). Any changes in the performance of the respiratory system, accompanying changes in ventilatory direction, could have significant effects on the fraction of total oxygen consumption ($\dot{M}o_2$) that is devoted to ventilation.

During forward ventilation, the water stream passes over the respiratory surfaces of the lamellae in a roughly counter-current direction with respect to the haemolymph flow within (Hughes et al., 1969). This counter-current flow enables a highly efficient exchange of respiratory gases between the external water and the haemolymph. However during reverse ventilation it is likely that this counter current flow is disrupted, potentially altering the effectiveness of gas exchange. McDonald et al. (1980) demonstrated that the efficiency of oxygen extraction (E_w%) was lower during periods of reverse ventilation than during adjacent periods of forward ventilation in *C. magister*. As a result, the convection requirement and metabolic cost of ventilation was increased when utilising reverse ventilation. Thus, although reverse ventilation may enable a buried crab to continue to successfully ventilate the branchial chambers, it is probable that the conditions for oxygen transfer across the gill will be altered. The effects of this have not been previously examined in a burrowing species.

The ability of the scaphognathites to generate ventilatory flow has also been shown to differ in the two modes in *C. magister*, with the scaphognathite stroke volume

(V_s) being lower in the reverse mode (McDonald et al., 1980). Again this would contribute to differences in the relative efficiencies of the two ventilatory modes.

In previous studies many of the observed patterns of ventilation shown by brachyuran species appear to be related to reducing this high ventilatory cost. For example, Burnett and Bridges (1981) suggest that periodic ventilatory pauses shown by resting *Cancer pagurus*, and many other species, serve to eliminate the high metabolic cost of ventilation altogether during periods of low oxygen demand. During a pause the oxygen requirements are met by utilising the venous reserve of oxygen in the blood. These stores can then be replenished when ventilation resumes. Utilising unilateral ventilation also appears to enable an animal to lower the ventilatory cost.

In *C. magister* during periods of low oxygen consumption, only one branchial chamber may be irrigated. This is accompanied by an increase in the efficiency of oxygen extraction on the ventilated side, enabling $\dot{M}o_2$ to be maintained despite a reduction in ventilation volume (McDonald et al., 1980). McDonald et al. (1977) also suggest that *C. magister* may lower the cost of ventilation during periods of high oxygen demand by reducing the incidence of events, such as brief reversals, that disrupt efficient countercurrent gas exchange.

Changes in ventilatory direction may affect the functioning of the respiratory apparatus of crabs in other ways. During forward ventilation, the action of the scaphognathites creates a negative pressure within the branchial chambers relative to the external medium. Conversely, during a period of ventilatory reversal, an increase in branchial chamber pressure (P_{branch}) to greater than ambient is seen. Taylor (1990) demonstrated that changes in gill transmural pressure (internal gill pressure - external pressure) *in vitro*, alter the vascular conductance of the compliant phyllobranchiate gill. An increase in P_{branch}, brought about by ventilatory reversal, would tend to compress the gills, restricting the flow of haemolymph. This could disrupt ventilation-perfusion matching at the gill and compromise oxygen uptake. Such an effect may be negligible in a species which only shows very brief reversals, but in a burrowing crab which reverse ventilates for long periods this may have significant consequences.

A series of muscles attaching between the dorsal roof of each branchial chamber and the dorsal carapace appear to be involved in regulation of haemocoelic pressure, these are the dorso ventral muscles (DVM). Contraction of the DVM compresses the large hepatic-branchiostegal sinus complex overlying the branchial chambers. Rajashekar and Wilkens (1991), working on *C. maenas*, found that DVM activity decreased in response to both spontaneous and artificial increases in P_{branch} and *vice versa*. Presumably relaxation of the DVM allows haemolymph, displaced from the gills by the pulse of positive P_{branch}, to be accommodated in the branchiostegal sinus without elevating haemocoelic pressure. In some amphibious species the

branchiostegite forms an auxillary site for oxygen uptake, and in fully terrestrial species the branchiostegites have developed into highly specialised pulmonary surfaces (Greenaway and Farrelly, 1990). Thus it is possible that the branchiostegites may also be alternative sites for gas exchange in aquatic reverse ventilating species, such as *O. catharus*.

This study therefore examines the relative costs of forward and reverse ventilation in buried and unburied *O. catharus*, in terms of oxygen uptake and ventilatory work and power, and uses this information to interpret observed patterns of ventilation. This is achieved by measuring oxygen uptake and ventilatory flow-pressure relationships in the two modes and burial states. The possibility of alternative sites of respiratory gas exchange is also examined using vascular corrosion casting techniques.

The Physiological Effects of Commercial Processes

Over 30 species of commercially important finfish, including Snapper, Rig and Grouper, are known to prey on *O. catharus*. Anecdotal evidence suggests that depletion of these finfish stocks over the last decade or so appears to have resulted in an increase in both the abundance and average size of paddle crabs. The increased effort required to catch dwindling numbers of traditional inshore finfish and shellfish species has meant that increased attention has been focused on other potential commercial species, such as *O. catharus*.

At present there is a relatively small and volatile fishery based on *O. catharus*. This has grown rapidly from less than a tonne in 1977 to 306 tonnes in 1985, and it appears that there is still considerable potential for the development of the paddle crab fishery. With potential habitat, (ie. sandy beaches) making up 53% of New Zealands 13 000km shoreline, and the large reproductive potential of *O. catharus*, the maximum sustainable yield for this fishery may be as large as, if not larger than, that for the red rock lobster *Jasus edwardsii*. However, there is only limited capacity for expansion of the domestic market and the key to realising this potential lies in development of larger overseas markets. The main potential markets for New Zealand crab products are the United States, Japan and South East Asia.

In the past, much of the catch was sold domestically to restaurants as whole fresh, cooked or frozen crabs. However, there is increasing demand in other sectors of the domestic market with crabs now being sold in supermarkets. As with other

shellfish species, such as mussels (*Perna canaliculus*) and *J. edwardsii*, live animals, assuring product freshness, are preferred. At present modest quantities of various crab products, including whole cooked and whole frozen crabs are being shipped to these markets.

To meet this local demand, live crabs are now routinely transported by air freight or road within New Zealand. Because of the problems associated with air freighting large volumes of water, commercially important marine animals are usually shipped dry. Distances in this country are relatively short, and animals are rarely emersed for periods longer than about 4 hours before reaching their destination. Live product is also highly sought after in the international marketplace for the same reasons. Because of New Zealands geographic isolation, long flights, often including stopovers en route, are required to get crabs to overseas destinations. As a result, the aerial exposure time for the animals is increased to 12-24 hours. This will present a major challenge to the respiratory system of a fully aquatic crab, such as *O. catharus*. The response of *O. catharus* to periods of aerial emersion has not been previously determined. Some trial international shipments have been made, but the survival of the crabs has been poor.

The respiratory and acid-base changes that occur during a number of stages of commercial handling and processing, including post capture recovery and experimental aerial emersion are examined. This information gives an indication the suitability of this species for live export. The information obtained from these experiments will hopefully assist in the development of a live export trade in *O catharus* from New Zealand.

Abbreviations Used

F_r	Ventilation Frequency (beats min^{-1})
$\dot{V}_w$	Ventilation Volume (l $kg^{-1}min^{-1}$)
$E_w\%$	Oxygen Extraction Efficiency (%)
P_iO_2	Inspired Oxygen Tension
P_eO_2	Expired Oxygen Tension
$\dot{M}O_2$	Oxygen Consumption (μmol $kg^{-1}min^{-1}$)
$\dot{V}_w/\dot{M}O_2$	Ventilatory Convection Requirement (ml μmol^{-1})
P_{branch}	Branchial Chamber Pressure (cm H_2O)
P_{trans}	Gill Transmural Pressure (cm H_2O)
V_s	Scaphognathite Stroke Volume (ml $beat^{-1}kg^{-1}$)
W_s	Stroke Work of Ventilation (J $beat^{-1}kg^{-1}x10^4$)
W_r	Ventilatory Power Output (mW kg^{-1})

CHAPTER 2

Ventilatory Behaviour of the New Zealand Paddle Crab Ovalipes catharus

Abstract

At rest, both buried and unburied *Ovalipes catharus* exclusively reverse ventilated. Periodic brief (3-5 sec.) reversals of ventilatory direction (coughs) were recorded from both groups. Mean branchial chamber pressure (P_{branch}) was significantly higher in the resting buried animals (1.47 ± 0.16 cm H_2O) than in the unburied crabs (0.26 ± 0.04 cm H_2O). Mean ventilation frequencies (F_r, left + right), were identical in the two groups (42 beats min^{-1}), and thus presumably the mean ventilatory flows were also similar. It is suggested that the observed difference in branchial chamber pressures result from added resistance to ventilatory water flow presented by the sediment surrounding buried animals. Changes in branchial resistance also appear to result from active regulation of the branchial water flow pathway. The most significant mechanism for such regulation is the ability to raise and lower the carapace dorsoventrally.

Fifteen minutes of continuous exercise by swimming did not significantly alter haemolymph pH in *O. catharus* but caused a significant increase in haemolymph [lactate] from 0.510 ± 0.057 to 1.480 ± 0.336 mmol l^{-1}. Exercise also induced periods of prolonged forward ventilation. Immediately following exercise, crabs that were prevented from burrowing spent a mean of 75.4 ± 14.2% of the time utilising forward ventilation, while crabs allowed to burrow into a sandy substrate following exercise spent only 32.5 ± 12.1 % of the time in the forward ventilatory mode. In addition, mean ventilation frequencies at this time were lower in animals allowed to burrow (forward, 185.2 ± 30.0; reverse, 198.9 ± 20.9 beats min^{-1}) than in crabs remaining unburied (forward, 272.5 ± 32.8; reverse, 281.5 ± 20.7 beats min^{-1}).

During forward ventilation, ventilatory water flow was similar to that described previously for several brachyuran species. Water entered the branchial chambers at the bases of the legs and flowed from the hypobranchial space, through the gill sieve to the epibranchial space, before being exhaled near the mouthparts. This flow is roughly countercurrent with respect to the direction of haemolymph flow within the gill lamellae. When the direction of flow was reversed, water entered anteriorly and passed directly into the epibranchial space. Much of this water passed over the dorsal surface of the gills before being exhaled posteriorly via large apertures between the 4th and 5th walking legs. Occasionally some water exited at the Milne-Edwards apertures indicating epi-hypobranchial flow, and the possibility of some concurrent gas exchange.

The implications of reverse ventilation and burial in soft sandy sediments for respiratory gas exchange and gill function are discussed.

Introduction

O. catharus is a swimming crab belonging to the family Portunidae. Swimming is achieved using the last pair of legs which bear flattened paddle-like dactyls. These paddles are also used for burrowing into the soft, sandy sediments on which this species is found. Individuals usually remain buried in the substrate for extended periods during the day, emerging mainly at night to feed on various species of molluscs and other prey.

A potential problem facing crabs buried in soft sediments, is the maintenance of a respiratory water current. In the brachyura, the gills lie within paired branchial chambers. Ventilatory water currents are drawn through these chambers by the action of the blade-like exopodites of the second maxillae (the scaphognathites). Typically water is drawn into the branchial chambers through apertures at the bases of the chelipeds and walking legs. This water passes into the ventral hypobranchial space lying between the gills and the thoracic wall. The water then flows between the gills to the dorsal epibranchial space and is pumped out of the branchial chambers anteriorly, through exhalent pores exiting near the mouthparts. This pattern of ventilation is referred to as the forward ventilatory mode.

Many decapod crustacean species, including *O. catharus*, periodically reverse the direction of ventilation (McLay & Osborne 1985; Arudpragasam & Naylor, 1964a, 1966; Caine, 1974; McDonald et al., 1980; Hartnoll, 1972; Batterton & Cameron 1978; McMahon & Wilkens 1977; Taylor 1984; Uglow 1973; Wilkens & McMahon 1972). This is brought about by reversing the direction of beating of the scaphognathites (Simmers & Bush, 1983). In general there are two main types of ventilatory reversals; brief and prolonged. Virtually all brachyurans and macrurans investigated to date show brief reversals of ventilation. These typically only last for 2 or 3 cycles of the scaphognathites, before forward ventilation is re-established. The function of these events is uncertain and may vary between species. Suggested roles in aquatic species include removal of accumulated detritus and irrigation of areas of the gill chambers that are poorly ventilated during forward ventilation (Arudpragasam & Naylor, 1964a & b, 1966; Hughes et al., 1969; Wilkens & McMahon, 1972). In terrestrial and semi-terrestrial species, reversals seem to be strongly involved with ventilation of pulmonary surfaces and retention of branchial water (Burggren et al., 1985; Eshky et al., 1990; Wilkens & Young, 1992; Maitland, 1992a). Sustained reverse ventilation is generally associated with aquatic species that burrow into soft sediments. In keeping with this trend, McLay & Osborne (1985) observed extended periods of reversed ventilation in *O. catharus* shortly following burial in sandy substrates. It is thought that reversing

the direction of ventilation enables buried animals to maintain a respiratory water stream from above the sediment surface. However, many burrowing species also exhibit prolonged periods of forward ventilation, particularly when unburied (Garstang, 1897a & b; Hartnoll, 1972; Caine, 1974; Taylor, 1984; Barshaw & Able, 1990).

In forward ventilating crabs, water and haemolymph move across the gill lamellae in opposite directions enabling the efficient counter-current exchange of respiratory gases between the external medium and the haemolymph (Hughes et al., 1969). Reversing the direction of ventilation disrupts these water flow patterns. This breakdown of counter-current flow has been shown to reduce the efficiency of oxygen extraction (E_w%) in *Cancer magister* (McDonald et al., 1980).

The action of the scaphognathites generates pressures in the branchial chambers (P_{branch}). During forward ventilation, the scaphognathites act as suction pumps drawing water through the branchial chambers. This creates negative pressures within the branchial chambers relative to the external medium. Alternatively, during a period of ventilatory reversal, the scaphognathites act as force pumps, pumping water into the branchial chambers. This results in an increase in P_{branch} to greater than ambient. Similar pressure changes have been shown to affect the perfusion and functioning of the delicate and compliant brachyuran phyllobranchiate gill *in vitro* (Taylor & Taylor, 1991).

Thus, changes in the direction of ventilation may have significant consequences for the performance of the respiratory system in crabs, especially those which show prolonged periods of both forward and reverse ventilation. In addition, burrowing itself may have an effect on respiratory function. McLay and Osborne (1985) made some cursory observations on the ventilatory behaviour of buried *O. catharus*. The present study was designed characterise the ventilatory behaviour of buried and unburied *O. catharus*, more fully. The proportion of time settled crabs spent utilising the two ventilatory modes in either burial state was examined, and the effects of exercise prior to burial on ventilatory behaviour were also investigated. This information will then be used as a basis for subsequent experiments to evaluate the physiological repercussions of reverse ventilation and burial.

Materials and Methods

Crabs of both sexes, weighing between 116 g and 454 g were collected from Pegasus bay and brought to the Zoology Department where they were kept in a recirculating sea water system at 15 ± 1°C, under a 12h day/12h night light cycle, for at least one week before being used in experiments. During this time they were fed twice weekly on freshly opened mussels (*Mytilus edulis* and *Perna canaliculus*) or lamb's heart. Ovigerous females and individuals with more than one missing appendage were not used. All animals were judged to be in the intermoult stage (stage C) of the moult cycle.

Resting Ventilation (Series I)

In order to monitor fluctuations in branchial chamber pressure (P_{branch}), resulting from ventilation, both branchial chambers were cannulated with portex polythene tubing (800/100/320; ID 1.14 mm, OD 1.57 mm). Each cannula was inserted ventrally, into the epibranchial space, through a hole drilled in the cuticle halfway between the base of the cheliped and the fifth antero-lateral spine of the carapace. The cannulae were fixed in place with cyanoacrylate cement then looped round and secured to the dorsal carapace with small strips of rubber. Each individual was allowed to recover for at least 48 hours before recordings were made. During this time, the open ends of the cannulae were plugged so that the normal respiratory water currents would not be disrupted.

Crabs were placed in a 17 l black plastic aquarium filled with aerated sea water at 15 ± 0.5°C. The aquarium was partially covered to reduce visual disturbance. For unburied crabs, the bottom of the experimental chamber was lined with a coarse plastic mesh. Individuals would grip the mesh, minimising spontaneous activity during recording. Animals used in the burial experiments were provided with 90 mm of washed beach sand. The water-filled branchial chamber cannulae were connected to Bell & Howell 4-327 blood pressure transducers. The signals from these were amplified by Gould 13-4615-58 universal amplifiers and the output displayed and recorded on a Gould 8188-2202-XX two channel thermal writing recorder. All individuals were allowed to settle for 5 hours in the experimental chamber before recording began. After this time, P_{branch} was monitored continuously for 1 hour. All recordings were made during the light phase of the day/night cycle.

Post Exercise Ventilation (Series II)

One branchial chamber was cannulated, as above. P_{branch} was used only as an indicator of the direction of ventilation. Bilateral ventilation rates (F_r), were monitored by impedance techniques. Using a dentists drill, a pair of holes were drilled on either side of the buccal frame, ventrally and dorsally with respect to the position of each scaphognathite. Small platinum wire electrodes were inserted through the holes and fixed in place with cyanoacrylate cement and rubber dam. It was assumed that, during bilateral ventilation, the direction of ventilation in the uncannulated chamber was the same as that on the cannulated side (no previous observations suggested the contrary). A small cork with a length of string attached was glued to the dorsal carapace of each animal. All crabs were left undisturbed overnight to recover from the operation.

The crabs were suspended, by the attached string, in fresh aerated sea water at 15 ± 1°C in an 80 l concrete tank. As noted by Booth & McMahon (1985), using the portunid *Callinectes sapidus*, suspension of the animals in the water column elicited spontaneous swimming behaviour. Using this method, all crabs were forced to swim continuously for 15 minutes. Some animals needed to be prodded occasionally so that they would swim for the entire period.

Using plastic 1 ml syringes fitted with 20 G needles, 500 μl prebranchial haemolymph samples were taken anaerobically, via the arthrodial membrane of the fifth leg of each crab, immediately prior to, and following, the exercise period. The pH of this sample was determined using an activon BJ332 flat bulb pH electrode thermostatted to 15 ± 0.5°C connected to a Phillips PW9415 ion-selective meter. A 100 μl subsample of haemolymph was deproteinated in 200 μl of ice cold 6% perchloric acid, snap-frozen in liquid N_2, and stored at -75°C for subsequent lactate analysis. A spectrophotometric enzymatic method was used to analyse the stored samples for lactate (Boehringer kit #139 084), with modifications suggested by Engel and Jones (1978). Changes in the absorbances of samples were read with a Kontron Uvikon 860 spectrophotometer.

Immediately after taking the postexercise haemolymph samples, each animal was transferred to the same experimental chamber used in experimental series I. The impedance electrodes were then connected to a two channel impedance coupler (Strathkelvin, Bioscience #A100 power supply), the signal from which was amplified and displayed through the same Gould amplifiers and recorder used above. The branchial chamber cannula from each animal was connected to a pressure transducer

and was flushed with sea water. The output from the transducer was amplified (Gould 13-4615-58 universal amplifier) and displayed on an ink writing flatbed recorder (BBC SE120).

Immediately following the exercise bout, and then at intervals throughout a 5 hour postexercise recovery period, bilateral scaphognathite rate (F_r, left + right) and unilateral P_{branch} were recorded continuously and simultaneously during 5 minute sample periods. From these samples, the percentage of time spent utilising each mode, reverse and forward F_r, the frequency of ventilatory pauses (F_{pause}) and the frequency of ventilatory coughs (F_{cough}) (see below), were calculated.

Branchial Water Flow Patterns

Large crabs (230 - 447 g) of both sexes, were used. A hole was cut in the branchial region of the dorsal carapace down to the epidermis overlying one branchial chamber, using a grinding wheel fitted to a dentists drill. After cutting, the animals were placed in crushed ice until they became torpid. A heated wire filament was then run along the cut made in the carapace to cut through and cauterise the underlying branchiostegite. The section of carapace and branchiostegite was removed and replaced with a piece of mylar sheet which was fixed in place with cyanoacrylate cement (Fig. 2.1). Only the posterior gills 6 to 9 were visible through the window as the hole could not be enlarged anteriorly without damaging the internal organs. Any attempt to do so resulted in death. Following a period of recovery, branchial water flow patterns were observed by pipetting trypan blue in 100% sea water near the inhalent apertures. The course of the dye through the branchial chambers could easily be seen through the window.

Data Analysis

All data are presented as mean ± 1 s.e.m. Comparisons between unpaired means were made by one-way ANOVA and tested at the 5% level of significance. Percentage data were transformed by using the arcsine square root transform to normalise the data and stabilise the variances. All analyses were calculated by the computer statistical software package BMDP SOLO version 2.0.

Fig. 2.1. A specimen of *O. catharus* that has been fitted with a branchial chamber window. The (7)th, (8)th and (9)th gills are visible through the window (see text for details).

Results

Burial Behaviour

The following account refers to the five pairs of pereiopods as a pair of chelipeds and four pairs of walking legs. The fourth pair of walking legs bear the flattened paddle-like dactyls.

All individuals in series I and II experiments that were allowed access to sand, burrowed into the sediment shortly after being introduced to the experimental chamber. At the initiation of burrowing the 4th pair of walking legs, bearing the flattened paddles, were dug into the sand at the rear, while the dactyls of the first

three pairs of walking legs were pulled down into the sand beneath the crab. The chelipeds were held either flexed against the pterygostomial region of the carapace or wide apart in an aggressive or defensive manner. A depression was formed beneath the animal by pushing sand forward with the flexed 1st and 2nd pairs of pereiopods. The animal would then step forward and drive the posterior margin of the carapace backwards, and at a downwards angle, into this depression. This was often repeated two or three times if the initial depression was not deep enough. Sand to the rear of the animal was brought into suspension by the action of the paddles and also apparently by pulses of exhaled water from the large branchial chamber apertures between the 4th and 5th legs. This aided the insertion of the body into the sediment by loosening the previously compacted sand, and suspended sand particles in the water, which fell forward helping to cover the animal. As burial was nearing completion, the animal would make small horizontal rocking movements causing sand to cover the dorsal carapace. When all movement had ceased, the chelipeds were tightly flexed against the front of the body. The 3rd maxillipeds were held closely against the prostomial region of the carapace, so that the inhalent water stream is filtered by the dense setae on the margins of the 3rd maxillipeds and carapace. When unburied, the 3rd maxillipeds are held in a lower position, separating the setose margins. Caine (1974) made similar observations on *O. guadulpensis*.

Contrary to the observations of McLay and Osborne (1985), several individuals remained partially exposed after the burrowing behaviour sequence had ended. The eyestalks and antennae were visible in most buried crabs, but varying degrees of exposure of the mouthparts, carapace and chelipeds were also observed. Insufficient depth of sand is unlikely to be a factor in this, as the depth used in the present study was 20mm greater than that used by the earlier workers. For analysis, all these individuals were considered to be fully buried. Some of the animals emerged briefly during the 5 hour settlement period, but were again buried when recording began.

Branchial Water Flow Patterns

Of the 9 gills in each branchial chamber only the four most posterior gills (6 to 9) were visible through the branchial window. Ventilatory flow routes in the region of gills 1 to 5 could not be observed, but inferences could be made from the timing and quantity of the appearance of dye at other sites. It should be noted that it is very difficult to accurately assess proportions of flow in dye studies, and that figures given are estimates only. In addition, the size of the branchial chamber apertures can be

altered by the crabs (see below), resulting in highly variable contributions of each aperture to total ventilatory flow.

In *O. catharus*, each branchial chamber has four apertures associated with the bases of the 5 pereiopods. These are bordered dorsally by the margin of the carapace and ventrally by the bases of the legs. Anteriorly, there is a large opening encircling the base of the cheliped. This is known as the Milne-Edwards (M-E) aperture. There is a smaller aperture between the bases of walking legs 1 and 2, and also between legs 2 and 3. Posteriorly, there is a another large opening between walking legs 3 and 4. The carapacial margin forming the apertures is fringed with dense setae, especially in the region of the large posterior openings. When a needle was used to stimulate the inner surfaces of the openings, the posterior margin of the carapace was drawn ventrally towards the limb bases, reducing the size of these apertures greatly. This lowering of the carapace occurred both unilaterally and bilaterally, so that the size of the apertures can be regulated independently. Carapace lowering also affected the size of the two lateral branchial chamber apertures between the 2nd and 3rd walking legs. Movement of the carapace seemed to occur about a pivot point that was situated anteriorly. As a result, the range of movement was greatest posteriorly, and thus the greatest degree of regulation was observed in the most posterior apertures.

In unburied individuals of *O. catharus* during forward ventilation, about 50 - 75% of the total inhalent flow entered the branchial chamber through the M-E opening (Fig. 2.2). All water entering this aperture appeared to pass into the ventral hypobranchial space between the gills and the thoracic wall. Approximately half of the inhaled dye was directed posteriorly in the hypobranchial space, staining the proximal regions of gills 7, 8 and 9 as it flowed between their lamellae and through to the dorsal epibranchial space. At times a small portion of the dye flowed around the posterior edge of gill 9, thus entering the epibranchial space without irrigating the gills. The remainder of the dye entering the M-E aperture could not be seen through the window, but rapidly appeared in the exhalent stream exiting the prebranchial apertures. It is likely that this portion of the stream irrigates the anterior gills 1 - 5, in a similar fashion to that suggested by Hughes et al. (1969) in *Carcinus maenas*.

The two openings at the bases of walking legs 1, 2 and 3 are of similar size and each contributed about 5% of the total inhalent flow. Water entering the aperture between the 1st and 2nd walking legs was initially directed into the hypobranchial space before irrigating gills 6, 7 and 8, proximally. The aperture between the 2nd and 3rd legs is posterior to the position of the 9th gill. As a result, some of the flow from this opening directly entered the epibranchial space. The remainder flowed into the hypobranchial space before irrigating the proximal regions of gills 7, 8 and 9. The remaining 15-25% of the total inhalent flow entered through the large posterior

opening between the 3rd and 4th legs. The pericardial organs lie postero-dorsally in the branchial chamber between the 9th gill and this aperture. All water entering the branchial chamber at this site flows directly into the epibranchial space. A large proportion of this stream flows over the dorsal surfaces of the gills. As it passes anteriorly, it mixes with the water coming up between the gills from the hypobranchial space. Some of the water entering the posterior openings flows ventrally beneath the gills and into the hypobranchial space. This flow irrigates the medial and distal portions of the posterior gills. Proximally, water is largely prevented from entering the hypobranchial space by the flow from the three anterior openings. Flow patterns in buried, forward ventilating animals could not be tested. However, measured increases in the interstitial Po_2 in the sand adjacent to the M-E apertures suggest that these openings remain the primary inhalent sites.

During reverse ventilation, the prebranchial apertures that lead to the scaphognathite pumping chambers become the inhalent apertures. Water directly enters the dorsal epibranchial spaces after leaving the prebranchial chambers. In unburied crabs at low values of F_r, it appeared that virtually all of the ventilatory water (> 95%) passed over the dorsal surfaces of the gills and was expelled through the large posterior openings between the bases of the 3rd and 4th walking legs. However, at high values of F_r particularly, some of the dye (approx. 5 - 10%) occasionally appeared out of the Milne-Edwards openings. Water exiting the posterior openings had a relatively high velocity, whereas water leaving the M-E apertures was moving very slowly. Dye was also injected directly into the hypobranchial space, during reversed ventilation, by inserting a 27 G hypodermic needle through the plastic window and between adjacent gill lamellae. The dye did not appear in the epibranchial space, indicating that there was no movement of water from the hypobranchial to the epibranchial space.

Following burial in sand, exhalent ventilatory streams could usually be seen welling up through the substrate, posterior to the position of the buried animal. This indicated that the animals were reverse ventilating. Exposing the posterior margin of the carapace revealed that the streams were issuing from the branchial chamber apertures between the 3rd and 4th walking legs. Smaller plumes could also be seen around the anterior and antero-lateral margins of the carapace. By excavating the sand covering the chelipeds, and 1st walking legs, it could be seen that the plumes originated from the M-E apertures, with most of the exhalent stream rising to the sediment surface between the inner face of the propodus of the cheliped and the pterygostomial region of the carapace (Fig 1.1). These spaces were first identified in *Bathynectes longipes* by Garstang (1897b) who referred to them as the "exostegal channels".

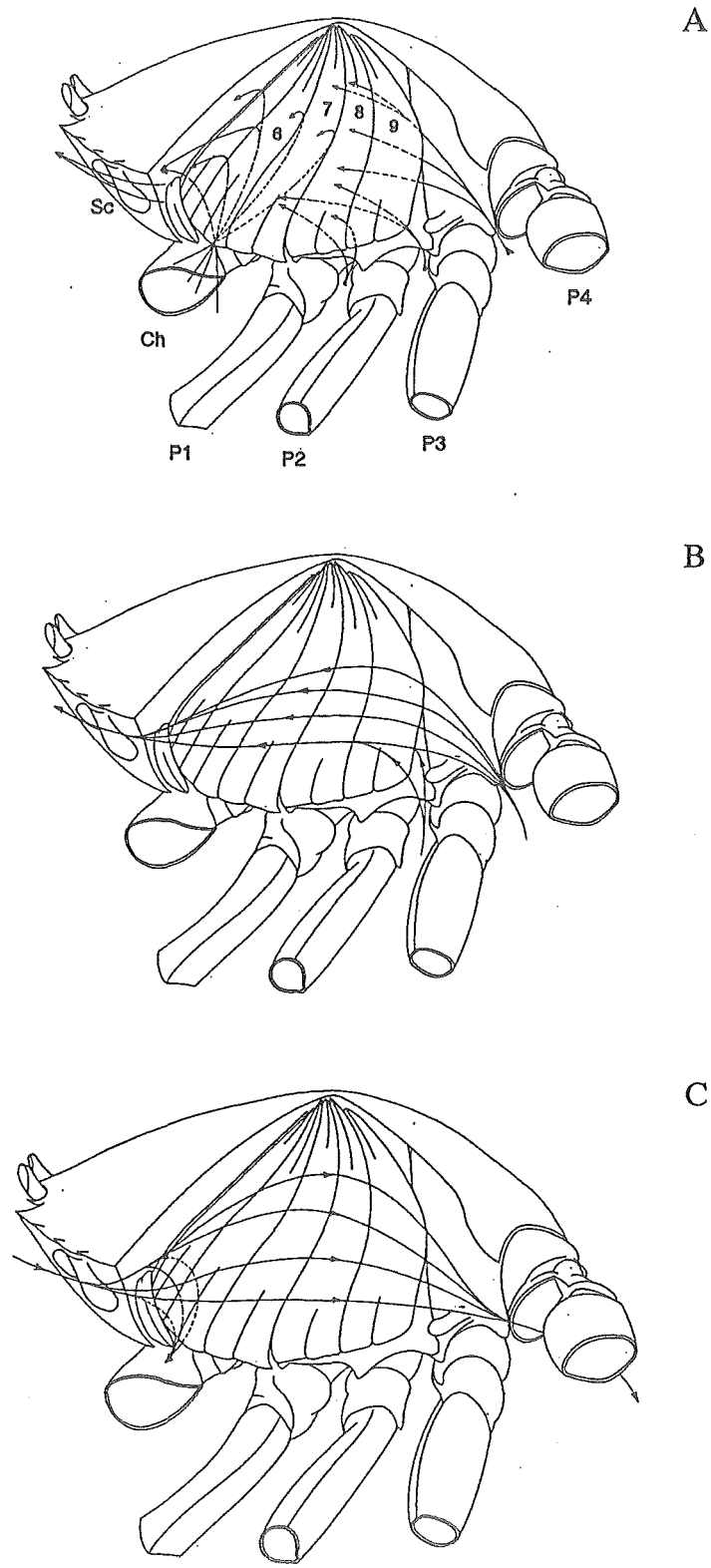

Fig. 2.2. Branchial water flow patterns in *O. catharus*. Flow patterns in the region of gills 6 to 9 were directly observed, whereas flow in the region of gills 1 to 5 is speculative. A) Hypobranchial flow during forward ventilation. B) Epibranchial flow during forward ventilation. C) Branchial flow patterns during reverse ventilation. Ch = cheliped, P1 - P4 = pereiopods 1 - 4, Sc = scaphognathite. ———— represents epibranchial flow and - - - represents hypobranchial flow.

Resting Ventilation (Series I)

At rest, both buried and unburied crabs exclusively ventilated in the reverse direction (Table 1). Prolonged periods of forward ventilation were not observed in either group. However, ventilation was regularly punctuated by very brief bursts of forward ventilation, each lasting between 3 - 5 seconds. I will refer to these events as "coughs". This term is descriptive only, and it is not intended to infer any specific function (see below). When both branchial chambers were being irrigated, coughs usually occurred bilaterally. In settled unburied crabs, the mean frequency of coughing (F_{cough}) was 14.6 ± 3.4 h^{-1}. In settled buried animals, a similar value of 10.8 ± 1.8 h^{-1} was recorded ($p > 0.05$). F_r (left + right) was unchanged with burial (41.5 ± 4.6 and 41.7 ± 6.5 beats min^{-1}, respectively ($p > 0.05$)). These were reverse ventilation frequencies only, as no forward ventilation was recorded. Despite comparable values of F_r, the mean P_{branch} of the buried individuals (1.47 ± 0.16cm H_2O) was significantly higher than in unburied crabs (0.26 ± 0.04cm H_2O) ($p < 0.05$).

Both buried and unburied crabs exhibited bi- and unilateral pauses, at rest. The frequency and duration of these events was similar between the two groups. Both types of pause occurred at a similar frequency within each group (approx. 0.5-1 h^{-1}) but unilateral pauses tended to have a much longer duration (20-25 minutes) than bilateral pauses (1-2 minutes).

Table 1.1. Summary of ventilatory behaviour of buried and unburied individuals of O. catharus at rest (* indicates a significant difference ($p < 0.05$); *t*-test). Data are presented as means ± 1 s.e.m.

	Unburied (n=9)	Buried (n=10)
Weight (g)	159.6 ± 17.6	176.6 ± 17.7
Time Spent Reverse Ventilating (%)	100 ± 0	100 ± 0
F_r (beats min^{-1})	41.7 ± 6.5	41.5 ± 4.6
P_{branch} (cm H_2O)	0.26 ± 0.04	1.47 ± 0.16*
F_{cough} (h^{-1})	14.6 ± 3.4	10.8 ± 1.8
$F_{bilateral\ pause}$ (h^{-1})	1.1 ± 0.5	0.6 ± 0.4
Bilateral Pause Duration (min)	1.3 ± 0.4	1.6 ± 0.4
$F_{unilateral\ pause}$ (h^{-1})	0.8 ± 0.3	0.6 ± 0.74
Unilateral Pause Duration (min)	26.2 ± 8.4	22.8 ± 4.8

Post exercise ventilation (series II)

During the exercise period, *O. cathanus* swam in a similar fashion to the portunid *Callinectes sapidus*, as described by Spirito (1972). Individuals propelled themselves sideways using the "sculling" action of the paddle-bearing legs. The first 3 pairs of walking legs were held out rigidly on the trailing side, but exhibited movements, similar to walking, on the leading side. The chelipeds were usually held flexed against the pterygostomial region of the carapace. However, during burst swimming, the trailing cheliped was also extended rigidly, while the cheliped on the leading side remained flexed. All crabs periodically rotated horizontally through 180° to lead with the other side, while continuing to move in the same direction. The frequency of this rotation appeared to increase with the frequency of leg movement, although this was not measured. Ghost crabs (*Ocypode spp.*) show similar rotation of the body when running (Herreid and Full, 1988). This may serve to distribute the work between the leg flexor and extensor muscle groups on both sides of the animal.

Following exercise, crabs that were allowed access to sand burrowed immediately, and remained buried for the entire 5 hour postexercise recovery period.

Mean haemolymph pH fell from 7.631 ± 0.065 (n = 15), at rest, to 7.466 ± 0.062 (n = 14) immediately after exercise. This small change was not significant ($p > 0.05$). However, haemolymph lactate concentration increased significantly from 0.510 ± 0.057 (n = 10) mmol l^{-1} to 1.480 ± 0.336 mmol l^{-1} (n = 10) over the same period ($p < 0.05$).

Immediately following exercise and burial in sand, crabs spent 32.5 ± 12.1% of the total time utilising the forward ventilatory mode (Fig. 2.2). This was significantly higher than that for the settled crabs (0%), but was significantly less than the proportion of forward ventilation shown by exercised crabs remaining unburied (75.4 ± 14.2%) ($p < 0.05$). Utilisation of the forward mode declined in both groups as recovery progressed. The mean proportion of time spent in forward ventilation was greater in unburied crabs at every sample time, but this difference was not significant at the end of the five hour recovery period ($p > 0.05$). After 5 hours of recovery, all but one of the buried crabs were continuously reverse ventilating, as were all but two of the unburied crabs.

Coughs were also recorded during periods of prolonged forward ventilation, however in these instances animals switched briefly to reverse pumping, rather than to forward pumping as in reverse ventilating animals.

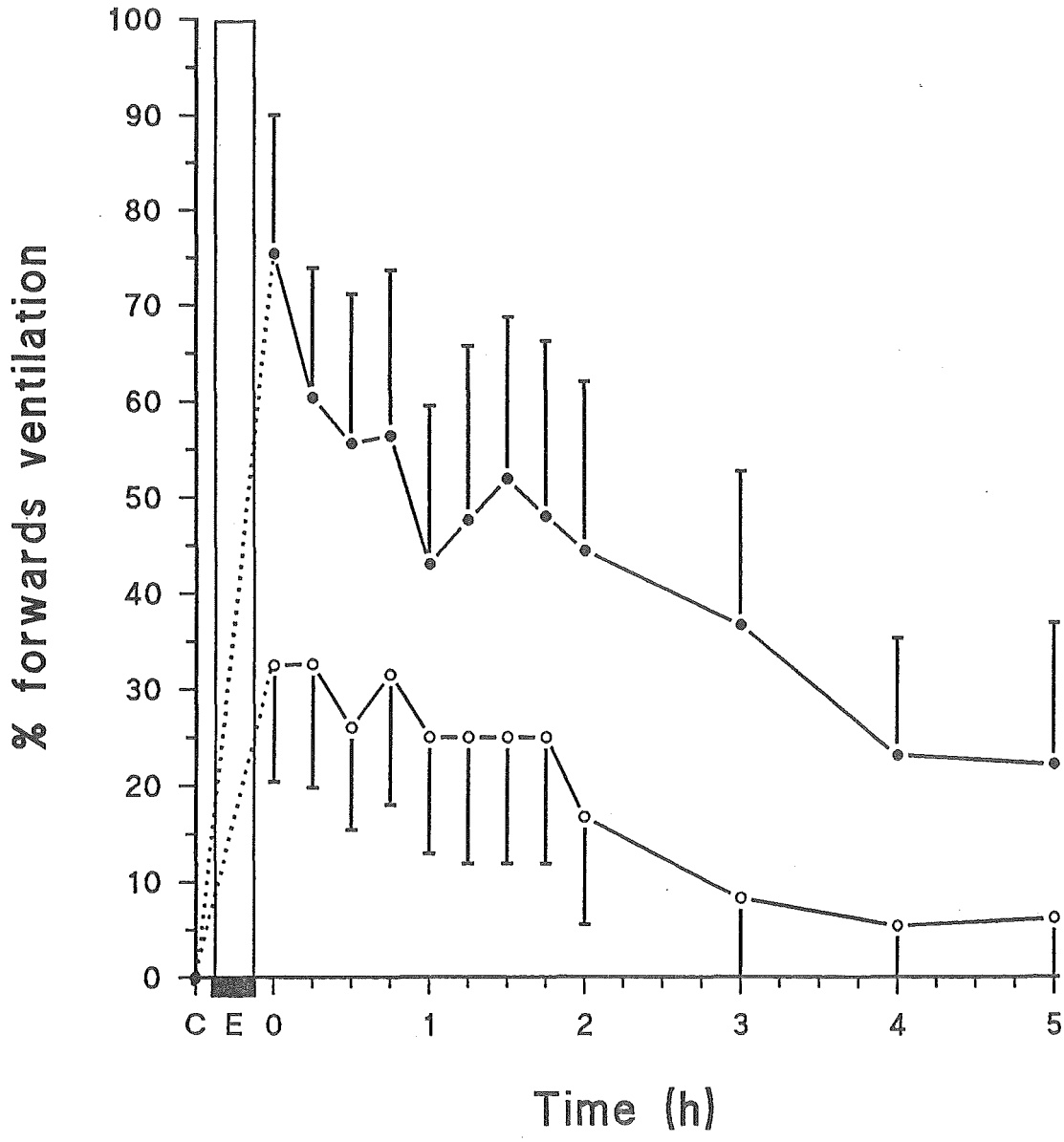

Fig. 2.3. The percentage of time spent utilising forward ventilation in buried (○) and unburied (●) *O. catharus* during recovery from 15 minutes of exercise by swimming. C = control value taken from resting animals, bar (E) represents the exercise period. Data presented as mean + or - 1 s.e.m.

Exercise stimulated a large increase in mean F_r (Fig. 2.4). Peak forward and reverse values of mean F_r were recorded from unburied crabs immediately following the exercise bout (272.5 ± 32.8 and 281.5 ± 20.7 beats min^{-1}, respectively). Crabs that were allowed to burrow into sand following exercise showed lower ventilation frequencies in either mode, with a maximum forward F_r of 185.2 ± 30.0 beats min^{-1}, and maximum reverse F_r of 198.9 ± 20.9 beats min^{-1}. Regardless of burial state, F_r declined rapidly, attaining values similar to those recorded from series I (settled) crabs between 1 and 2 hours following exercise. After 5 hours recovery, mean reverse F_r in unburied and buried crabs was 51.2 ± 10.0 and 36.6 ± 12.4 beats min^{-1}, respectively. These values were not significantly different from those of the settled animals ($p > 0.05$) (Table 1.1).

A number of unburied crabs were spontaneously active during recovery. This tended to obscure the postexercise pattern of ventilatory behaviour by elevating F_r and, in some instances, triggering periods of forward ventilation in animals that were reverse ventilating. For these reasons, excessively active animals were rejected.

F_{cough} was highly variable between sample times and there was no apparent relationship between F_{cough} and F_r in buried or unburied crabs, utilising either mode (Fig 2.5). Buried crabs showed a sharp decline in F_{cough} following exercise, whereas crabs remaining unburied showed a small increase in F_{cough}.

Brief unilateral pauses were recorded from only three of the postexercise animals (1 buried, 2 unburied) near the end of the 5 hour recovery period.

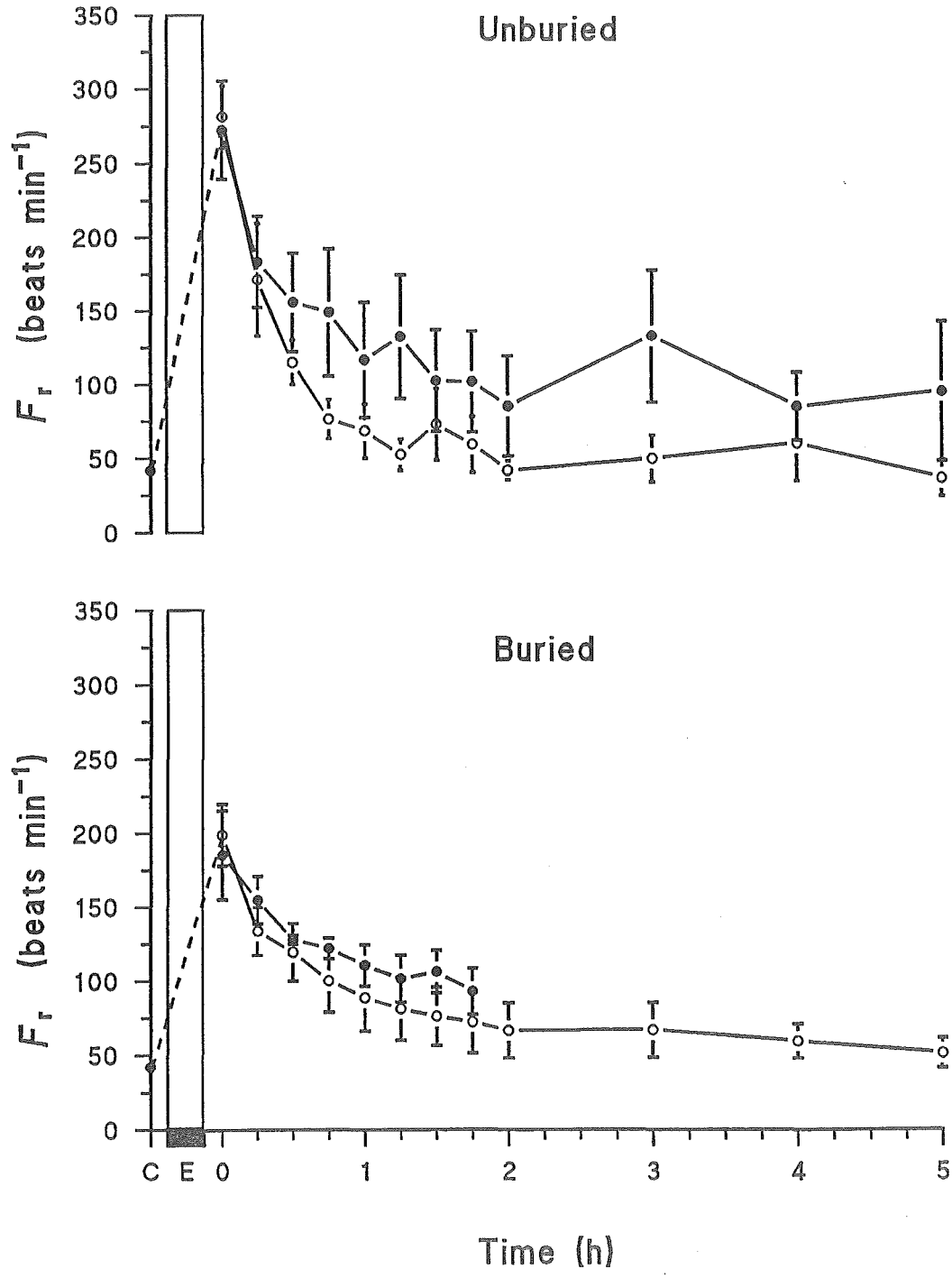

Fig. 2.4. Changes in ventilation frequency (F_r) in buried and unburied *O. Catharus* following 15 minutes of continuous exercise by swimming. ● = forward F_r, ○ = reverse F_r. C = control value taken from resting animals, bar (E) represents the exercise period. Data presented as mean ± 1 s.e.m.

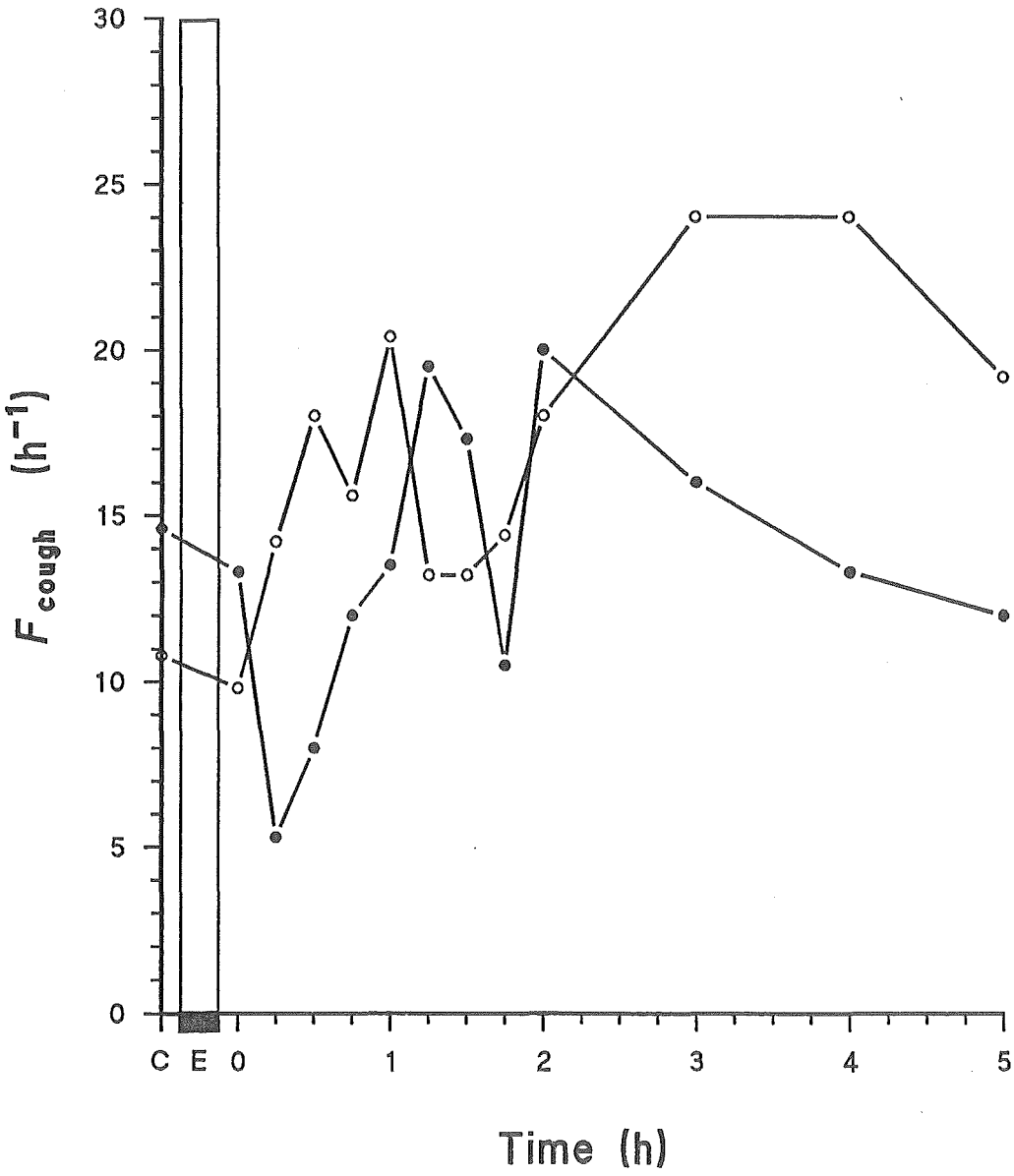

Fig. 2.5. Changes in frequency of coughs (F_{cough}) in buried (●, n = 11) and unburied (○, n = 9) *O. catharus* during recovery from 15 minutes of exercise by swimming. C = control value taken from resting animals, bar (E) represents the exercise period. Data presented as means only due very large standard errors.

Discussion

Ventilatory Behaviour

The present study confirms an earlier suggestion that *Ovalipes catharus* reverse ventilates when buried in sandy substrates (McLay and Osborne, 1985). Reverse ventilation accompanying burial is seen in a number of sand-burrowing brachyuran species. Interestingly, apart from two highly specialised burrowers *Atelecyclus rotundatus* (Taylor, 1984) and *Corystes cassivelaunus* (Arudpragasam and Naylor, 1966; Hartnoll, 1972) which form breathing tubes with their highly setose antennae, portunid species, including two other members of the *Ovalipes* genus, make up the remainder of the accounts of burrowing reverse-ventilating crabs in the literature. These are *Bathynectes longipes*, *Portumnus nasutus* (Garstang, 1897a, 1897b), *Carcinus maenas* (Taylor and Butler, 1973), *Ovalipes guadulpensis* (Caine, 1974), and *Ovalipes ocellatus* (Barshaw and Able, 1990). Many of these authors suggest that reverse ventilation enables a buried animal to obtain a relatively well oxygenated and sediment free inhalent water stream from above the sediment surface, as the mouthparts and prebranchial apertures are generally closest to the sediment surface when buried. Forward ventilation would require utilising presumably oxygen poor interstitial water (however, no measurements of interstitial oxygen tensions were made in these studies). Several of the studies however, also show that animals may bury so deeply, that they have no contact with the sediment surface, or free water. For example, Barshaw and Able (1990) noted that the anterior inhalent region of buried *O. ocellatus* was an average of approximately 5 cm below the sediment surface. *Ovalipes guadulpensis* was, at times, covered entirely with up to 2 cm of sand (Caine, 1974). *C. cassivelaunus* and *A. rotundatus* are able to burrow deeply into the substrate and maintain contact with the sediment surface via specialised antennal breathing tubes (Hartnoll, 1972). However, when the inhalent current at the tip of the tube was tested, it was found to be not as strong as expected, leading to the suggestion that interstitial water may be drawn through the setae along the length of the tube. Some specimens buried so deeply that the tube could not be seen at all. Presumably such deeply buried individuals of all these species obtain their inhalent ventilatory stream by exclusively filtering interstitial water. If interstitial oxygen tensions are low enough to compromise oxygen uptake, it would be seem to be disadvantageous for deeply buried animals to further hinder efficient oxygen uptake by reverse ventilating, and disrupting counter-current gas exchange at the lamellar surfaces (McDonald et al., 1980). In the

present study, interstitial oxygen tensions were found to be as low as 50% of ambient (Chapter 3). However, by forming the exostegal channels, buried forward ventilating *O. catharus* can draw a normoxic inhalent water stream from above the sediment surface and can maintain levels of oxygen consumption ($\dot{M}o_2$) comparable to those found in unburied animals (Chapter 3). Thus the exostegal channels enable buried animals to maintain contact with the superincumbent water as do the antennal tubes shown by *A. rotundatus* and *C. cassivelaunus*.

Most brachyurans show prolonged utilisation of one ventilatory mode only, typically this is the forward mode. Aside from "coughs", the ventilatory responses shown by these species to variations in external and internal variables, are generally restricted to changes in F_r. However, species such as *O. catharus*, that are able to ventilate continuously in both forward and reverse direction, exhibit more complex responses. Not only can ventilation frequency be altered, but the direction and proportion of time spent in each mode is also variable. Many burrowing species show different patterns of ventilation when they emerge from the sediment. For example, *A. rotundatus* predominately forward ventilates when unburied and also when partially buried (ie. the exostegal channels are visible). Prolonged reverse ventilation is only utilised upon deep burial (Taylor, 1984). Approximately equal proportions of time are spent in the two modes in unburied individuals of *C. cassivelaunus* (Hartnoll, 1972), while unburied *O. guadulpensis* utilise both modes, with reverse ventilation still predominant Caine (1974). Well settled specimens of *O. catharus* exclusively reverse ventilated, regardless of burial state. But activity was found to initiate periods of forward ventilation, and from casual observations, it appeared that the amount of time spent forward ventilating was dependant on the duration and intensity of the exercise bout. It is possible that the forward ventilation that Caine (1974) observed in unburied specimens of *O. guadulpensis* also occurred in response to spontaneous activity. Unfortunately there was no reference to the activity state of the animals in this study. However, the ventilatory behaviour in these two closely related species seems to be very similar. It appears that utilisation of the two ventilatory modes in buried and unburied animals may be associated with the relative cost of each mode. For example, in unburied *O. catharus*, the convection requirement ($\dot{V}_w/\dot{M}o_2$) is higher during reverse ventilation than when utilising the forward mode, especially at high values of $\dot{V}_w$ (Chapter 3). This is largely due to the breakdown of countercurrent exchange during reverse ventilation. As a result, unburied crabs utilised the forward mode predominately, during the period of increased oxygen demand following exercise. However, upon burial, $\dot{V}_w/\dot{M}o_2$ is similar in the two modes, but there is a large increase in the work and power requirements of generating flow in the forward mode (Chapter 3), and in buried crabs reverse ventilation becomes the preferred

mode, regardless of oxygen demand. It appears that ultimately reverse ventilation is the preferred mode in *O. catharus* regardless of burial state, as the proportion of time spent in ventilating in this direction increases with settlement, until it is utilised exclusively by both buried and unburied crabs. It should be noted that in the wild, *O. catharus* would not be expected to remain quiescent above the sediment surface. Individuals only emerge from the sediment for specific purposes, such as predator avoidance, migration and feeding, etc. Thus, the observed ventilatory behaviour of resting unburied crabs must be considered with this in mind. There would be little, or no pressure to adapt a ventilatory strategy to being unburied while at rest. The observed behaviour of experimental crabs in this situation may simply be the inappropriate utilisation of the only resting ventilatory response available to the animal.

The prolonged increase in mean F_r and increased haemolymph [lactate] following exercise in *O. catharus*, indicate that an oxygen debt was incurred during the period of activity. Similar increases in haemolymph [lactate] in buried and unburied crabs suggest that the severity of the exercise bout was comparable. Thus postexercise $\dot{M}o_2$ would be initially elevated to a similar level in both groups. Despite this, postexercise F_r was substantially lower in buried animals (Fig. 2.4), suggesting a smaller ventilation volume ($\dot{V}_w$). This reduction in ventilatory flow does not appear to prolong recovery in buried animals, due to higher oxygen extraction efficiencies shown by this group (Chapter 3).

Brief reversals of ventilation, or "coughs", are seen in many decapod species, both burrowers and non-burrowers. Two principal functions have been suggested for these events: a) gill cleaning and removal of accumulated detritus and b) oxygenation of areas of the branchial chamber that are poorly irrigated during forward ventilation, in particular the posterior gills. These were the main conclusions of Arudpragasam and Naylor (1966), who investigated patterns of gill ventilation in three brachyurans (*C. cassivelaunus*, *Cancer pagurus* and *Macropipus puber*) and one macruran (*Homarus gammarus*). Coughs appeared to have a significant gill cleaning function in *C. cassivelaunus*, *C. pagurus* and *H. gammarus*, as the addition of carmine particles to the water resulted in an increase in the frequency of these events. This treatment had little effect on *M. puber*. However, both *M. puber* and *C. pagurus* showed an increased cough frequency in response to artificial closure of the posterior branchial apertures, supporting the suggestion that coughs enabled effective irrigation of the posterior gills. Of the three brachyuran species, only *C. pagurus* took in an appreciable volume of respiratory water between the bases of the last pair of perieopods. As a result, the posterior gills in this species are well irrigated during forward ventilation. This was correlated with a much lower frequency of coughing (8 - 10 hour^{-1}) than in *M. puber*

(25 - 30 hour^{-1}). All of Arudpragasam and Naylor's observations were made on forward ventilating species whereas *O. catharus* is a predominately reverse ventilating species. Given that the branchial water flow patterns are quite different in the two modes it is difficult to make comparisons with the previous study. This is complicated by the variability seen in water flow patterns which appear to largely result from the opening and closing of the posterior branchial apertures produced by dorso-ventral movements of the carapace. However, the frequency of coughing was highly variable in resting unburied reverse ventilating *O. catharus* (6 - 34 hour^{-1}) and it may be possible that this variation is related to the opening and closing of the posterior branchial apertures (see below). The frequency of coughs may increase when the size of the posterior apertures is reduced and irrigation of the posterior gills is diminished. A change in ventilatory direction regardless of the predominant mode, necessitates a rebreathing of previously expired water which will be oxygen depleted. Thus it seems unlikely that the posterior gills would benefit from being irrigated with such water.

Coughs were often evoked in *O. catharus* in response to obvious chemical or olfactory stimuli, supporting the suggestion that coughs may have a gill cleaning function in this species. Coughs were frequently initiated when pipetting solutions of trypan blue or milk into the inhalent stream and were often accompanied by several rapid retractions of the eyestalks and a sculling motion of the 3rd maxillipeds. Milk seemed to elicit a feeding response, as seen when feeding the crabs with mussels. After the addition of milk, individuals moved towards the source of the stimulus with the chelipeds extended, presumably trying to grasp the potential food item. Whereas trypan blue appeared to be irritating to the crabs and reversing the direction of ventilation expelled the solution from the branchial chambers and buccal area. No attempt was made by these animals to approach the source of the solution. Similar responses have been demonstrated in other species to matter, such as carmine or charcoal particles introduced into the inhalent ventilatory stream (Garstang, 1897b; Berlind, 1975; McDonald et al., 1977). The sudden increase in P_{branch} associated with coughs during prolonged forward ventilation may serve to compress the gills briefly, enabling previously trapped material to dislodge and be expelled (Taylor et al., 1992). Obviously this would not apply to coughs shown by *O. catharus* when continuously reverse ventilating as these events are normally accompanied by a sudden reduction in P_{branch}.

McDonald et al. (1977) noted that coughs in *Cancer magister* often appear to occur in the absence of any extrinsic stimuli and suggest that these events may simply result from a pre-programmed motor output from the CNS. *O. catharus* exhibits coughs during both sustained forward and reverse ventilation. Given that ventilatory water flow pathways and P_{branch} effects would be quite different in the two modes, it

seems unlikely that coughing would have an identical gill cleaning or gas exchange function in both modes. Instead they may partly reflect this innate rhythm.

McDonald et al. (1977) also observed a reduction in F_{cough} in *C. magister* during periods of high oxygen demand. McMahon and Wilkens (1977) identified a similar pattern of ventilation in *C. productus*. Initially animals showed high rates of forward ventilation with very few coughs. As F_r declined, the incidence of brief reversals increased and eventually uni- and bilateral pauses occurred. Coughs would be expected to interfere with the uptake of oxygen at the gills, through disruption of counter-current exchange. McDonald et al. suggested that by reducing the incidence of these disruptive events, an animal may reduce the cost of ventilation during periods of high oxygen demand. Presumably the coughs shown by unburied *C. pagurus* have a nonrespiratory function which would have to be maintained at the expense of "optimal" gas exchange conditions, in fact the overall effectiveness of the system may depend on such events to prevent clogging of the gills with detritus. No clear relationship between cough frequency and F_r was obvious in either buried or unburied individuals of *O. catharus* following exercise.

As discussed, *O. catharus* is able to regulate the size of the branchial chamber openings by raising and lowering the dorsal carapace. Arudpragasam and Naylor (1966) suggested a similar ability in *C. pagurus*, but no indication was given of a possible mechanism for this. More recently, Maitland (1992a, 1992b) identified regular, cyclical depression and elevation of the carapace in the semi-terrestrial ocypodid *Holoecious cordiformis*. These movements were termed "carapace pumping", and were shown to be associated with aerial ventilation of the pulmonary surfaces. Maitland also found that dorsoventral carapace movement was synchronised closely with changes in the direction of beating of the scaphognathites. In subsequent experiments (Chapter 3), cannulae were inserted into the branchial chambers of *O. catharus* through holes drilled in the posterior carapace. These were to enable the withdrawal of branchial water samples. Often in buried animals, these cannulae were felt to move sharply downward, with no obvious movement of the sand overlying the buried crab. It was found from recordings of $\dot{V}_w$, that these movements were synchronised with coughs (ie. brief switches to forward ventilation). Forward ventilation generates a negative P_{branch} which, in buried animals, would tend to suck sand particles into the branchial chambers. By lowering the carapace during forward ventilation, the size of the posterior openings may be reduced, and the setose margins of these apertures would be brought together, preventing this. Partial closure of the branchial apertures would increase flow resistance and could partially account for the increased branchial chamber pressures recorded from buried crabs. The sediment in which an animal is buried would also serve to increase resistance to flow. Wilkens and

McMahon (1972) identified similar movements of the carapace in the lobster *Homarus americanus*. These reflex movements were closely synchronised with brief ventilatory reversals and were caused by contraction of the epimeral attractor muscles. It would be interesting to make EMG recordings from the epimeral attractors in buried forward ventilating *O. catharus* to see if there is an increase in the activity of these muscles over that shown by reverse ventilating individuals.

Previous studies (McDonald et al., 1977) have shown that, in brachyurans, ventilatory pauses are absent during periods of high F_r and begin to appear only when animals are well settled. This was the pattern observed following exercise in both buried and unburied *O. catharus*. Unilateral and bilateral ventilatory pauses are thought to enable animals to periodically reduce or eliminate the high cost of ventilation during periods of low oxygen demand (Burnett and Bridges, 1981, McDonald et al., 1980).

Branchial Water Flow Patterns

The branchial water flow patterns in unburied, forward ventilating *O. catharus* are very similar to those found in *C. maenas* (Hughes et al., 1969). Most of the ventilatory stream flows from the ventral hypobranchial space, between the gill lamellae, and up into the dorsal epibranchial space. Internally, haemolymph enters the gill from the systemic circulation via the dorsal afferent vessel, and flows through the gill lamellae to the ventral efferent vessel. Thus, the general directions of water and haemolymph flows across the gills are counter-current. This arrangement enables the Po_2 of haemolymph leaving the gill to approach that of the external medium (P_io_2)(Dejours, 1975). General similarities of the gross morphology of the gills and branchial chambers of aquatic brachyurans and the relatively high oxygen extraction efficiencies found in many species (McMahon and Wilkens, 1983) suggest that the counter-current mode of gas exchange predominates in this group. Some epi-hypobranchial flow was observed in reverse ventilating animals. This would establish roughly concurrent water and blood flows at the gills. Concurrent exchange is not as efficient as countercurrent (Dejours, 1975). The Po_2 of haemolymph leaving such a system would be approximately the average of the Po_2 of the venous haemolymph entering the gill and the P_io_2, depending on any limitation on diffusion. However a recent study (Richards, 1992) suggests that, at the level of individual lamellae, haemolymph flow in *O. catharus* may be more cross-current with respect to the direction of external water flow. Cross current exchange is of intermediate efficiency

when compared to cross- and concurrent models (Dejours, 1975). If the gills of *O. catharus* are acting as crosscurrent gas exchangers, regardless of ventilatory direction, the impact of changes in ventilatory direction on lamellar gas exchange may be reduced.

Like *C. maenas*, unburied, forward ventilating *O. catharus* take a significant amount of ventilatory water in through large posterior openings between the 3rd and 4th walking legs. In both species, most of this water enters the epibranchial space directly and passes anteriorly over the dorsal surface of the gills. Hughes et al. (1969) noted that this water remained relatively well oxygenated, indicating that oxygen extraction from this part of the ventilatory stream is poor. This serves to reduce the overall oxygen extraction efficiency. Not all species possess the large posterior openings, for example ventilatory flow in *Macropipus puber* is virtually halted when the M-E apertures are experimentally occluded (Arudpragasam and Naylor, 1966). The posterior apertures are also absent in *Cancer novazealandiae* (pers. obs.).

Arudpragasam and Naylor (1964a) suggest that a large proportion of the ventilatory stream flows over the dorsal surfaces of the gills in *C. maenas* during reverse ventilation. Direct observations in *O. catharus* again show a similar pattern of flow. Most of the inhaled water appears to flow through the epibranchial space only, before exiting via the posterior openings. This water stream appears to largely bypass the respiratory surfaces. However, the oxygen extraction efficiency (E_w%) in reverse ventilating animals remains reasonably high (Chapter 3), suggesting that either the lamellae are better irrigated than first thought, or that alternative sites for gas exchange, such as the branchiostegites are being utilised (Chapter 4).

During reversed ventilation in unburied *O. catharus* some water occasionally exits the branchial chambers via the M-E apertures. It is probable that water leaving the prebranchial pumping chambers at a high velocity is forced between the anterior gills and into the hypobranchial space, however direct observations of flow patterns in this region of the branchial chamber could not be made. This might explain why proportionally more water exited the M-E openings at higher ventilatory flow rates, when the velocity of the ventilatory stream is greater. Following burial, a greater flow of water from the M-E apertures is generally seen. This may result from a partial closure of the large posterior branchial apertures via depression of the carapace (Chapter 3). All water exiting the M-E apertures must presumably pass from the epibranchial space to the hypobranchial space via the inter-lamellar spaces. This would establish roughly co-current flows of haemolymph and water across the gills when ventilation is reversed.

Chapter 3

The Energetic Cost of Ventilation in the New Zealand Paddle Crab Ovalipes catharus.

Abstract

In unburied crabs, values of oxygen extraction efficiency (E_w%) were significantly lower and values of the convection requirement for ventilation $\dot{V}_w/\dot{M}o_2$ were significantly higher during periods of reverse ventilation than during adjacent periods of forward ventilation. This was presumably due to disruption of gill irrigation when ventilation is reversed. However, in buried crabs E_w% and $\dot{V}_w/\dot{M}o_2$ were similar in the two modes. Thus, in buried animals it would appear that the forward ventilatory mode is as effective as the reverse mode for oxygen uptake.

Branchial chamber pressures (P_{branch}) in buried animals were much higher than in unburied crabs. Mean values of P_{branch} in unburied forward and reverse ventilating animals were similar in magnitude but of opposite sign (-1.67 cm H_2O at mean $\dot{V}_w$ = 0.54 l $kg^{-1}min^{-1}$; 1.44 cm H_2O at mean $\dot{V}_w$ = 0.45 l $kg^{-1}min^{-1}$, respectively). Mean P_{branch} was significantly greater in buried crabs utilising reverse ventilation (2.70 cm H_2O, mean $\dot{V}_w$ = 0.27 l kg^{-1} min^{-1}). However, when forward ventilating, buried animals showed significantly greater pressures than all three other groups (mean P_{branch} = -7.2 cm H_2O, mean $\dot{V}_w$ = 0.28 l $kg^{-1}min^{-1}$). The higher values of P_{branch} recorded from buried crabs may result from the added resistance to ventilatory water flow presented by the sediment in which they were buried. Active regulation of branchial chamber resistance by the animals may also have an effect. As a result of the higher values of P_{branch}, stroke work (W_s) and ventilatory power requirements (W_r) were increased when buried, especially when utilising the forward ventilatory mode.

In forward and reverse ventilating unburied animals the mean stroke volume of the scaphognathite pump (V_s) was 4.86 ± 0.14 ml $beat^{-1}kg^{-1}$ and 4.92 ± 0.15 ml $beat^{-1}kg^{-1}$, respectively, while mean forward and reverse V_s in buried crabs was 3.63 ml ± 0.14 $beat^{-1}kg^{-1}$ and 4.11 ml ± 0.14 $beat^{-1}kg^{-1}$, respectively. The reduced values of V_s in buried animals are consistent with the suggestion that ventilatory resistance is increased when buried in soft sandy sediments.

The net effect of these differences in $\dot{V}_w/\dot{M}o_2$, P_{branch} and V_s were to alter the cost of ventilation, as a fraction of total $\dot{M}o_2$, in the four treatment groups. At a $\dot{V}_w$ of 0.6 l $kg^{-1}min^{-1}$, estimates of the ventilatory fraction of total $\dot{M}o_2$ were as follows: In unburied crabs 12.1% of total $\dot{M}o_2$ was devoted to ventilation when utilising the forward mode. This cost increased to 23.6% when ventilation was reversed. However, in buried crabs forward ventilation was more energetically expensive requiring 43.3% of total $\dot{M}o_2$ while reverse ventilation required only 20.0%. It is suggested that the increased dependance on reverse ventilation shown by burrowing crab species is related to lowering the high cost of ventilation when buried by reducing utilisation of the more energetically expensive forward mode.

Potential mechanisms of control of ventilatory switching are discussed.

Introduction

In the brachyura, the gills lie within paired branchial chambers. Aquatic species draw ventilatory water currents through these chambers by the rhythmic beating of projections of the second maxillae, the scaphognathites. Generally, water is drawn into the branchial chambers through apertures at the bases of the chelipeds and walking legs, and is exhaled anteriorly, through pores exiting near the mouthparts. This pattern of ventilation is regarded as normal forwards ventilation. Many decapod crustacean species, including brachyurans and macrurans, also have been shown periodically to reverse the direction of ventilation. Typically, these reversals are very brief, lasting perhaps 2-3 cycles of the scaphognathites. Reversals are thought to help prevent the accumulation of detritus on the gills by backflushing the ventilatory water pathways (Arudpragasam and Naylor, 1966; Berlind, 1977, McDonald et al., 1977). Crabs which burrow into soft sediments tend to show much longer reversals, particularly when buried, so that reverse ventilation is frequently the primary ventilatory mode in these species (Hartnoll, 1972; Caine, 1974; Taylor, 1984; Barshaw and Able, 1990). This has also been shown to be the case in *O. catharus* (McLay and Osborne, 1985; chapter 2). Most previous studies suggest that reversing the direction of ventilation enables buried animals to maintain the ventilatory stream by drawing water from above the sediment surface. Futhermore, Caine (1974) suggests that reversal of ventilation in buried *Ovalipes guadulpensis* precludes the utilisation of presumably oxygen poor interstitial water for ventilation. However, observations of branchial water flow patterns in *O. catharus* reveal that superincumbent water is used for ventilation during both forward and reverse ventilation in buried crabs (Chapter 2). This is enabled when forward ventilating by the formation of the exostegal channels, first observed by Garstang (1897a) in *Portumnus nasutus*. Given this observation, the increased dependance on reversed ventilation seen in buried crabs remains to be explained and requires further investigation.

Changes in the direction of ventilation may affect respiratory function in brachyurans in a number of ways. For example, during normal forward ventilation, the ventilatory water stream passes over the gas exchange surfaces of the gills in a roughly countercurrent direction to the haemolymph flow within the gill (Hughes et al., 1969). This permits a highly efficient exchange of respiratory gases between the haemolymph and the external water (Dejours, 1975). Upon reversal of ventilation, the countercurrent patterns of water and blood flow break down (Hughes et al., 1969; Chapter 2). This reduces the effectiveness of gas exchange at the gill. McDonald et al. (1980) showed that the convection requirement ($\dot{V}_w/\dot{M}o_2$) in unburied *Cancer magister* increased by approximately 50% when the direction of ventilation was

reversed. Thus, a greater volume of water must be pumped per unit of oxygen consumed, increasing the energetic cost of ventilation when utilising this mode.

The action of the scaphognathites generates pressure within the branchial chambers. During forward ventilation, the scaphognathites act as suction pumps drawing water out of the branchial chambers. This creates negative branchial chamber pressures (P_{branch}) relative to the external medium. Alternatively, during a period of ventilatory reversal the scaphognathites pump water into the branchial chambers, resulting in values of P_{branch} that are greater than ambient. The magnitude of the P_{branch} generated is proportional to both the rate of ventilatory flow and the resistance of the branchial water flow pathway (ie. the resistance to flow presented by the gill sieve and branchial chamber apertures). In producing flow against the branchial resistance, the musculature of the scaphognathites must perform work. The amount of oxygen consumed by these muscles is proportional to the work of ventilation. Water is a dense medium, and largely because of this the work required for ventilation in aquatic animals is comparatively high. The fraction of the total $\dot{M}o_2$ devoted to ventilation in brachyurans has been estimated at as much as 30% in the shore crab *Carcinus maenas* (Wilkens et al., 1984). P_{branch} in settled buried *O. catharus* was found to be significantly greater than in unburied individuals (Chapter 2). The sediment in which an animal is buried may increase the total resistance to ventilatory flow, thereby increasing the P_{branch}, ventilatory work, and cost of ventilation.

Experiments were carried out to investigate the effects of burial in sand on ventilation in *O. catharus*. The relative energetic cost of ventilation (ie. ventilatory fraction of $\dot{M}o_2$) in the two modes in buried and unburied crabs was also examined to try to explain the observed increased dependance on reverse ventilation seen in buried individuals in Chapter 2. This was achieved by measurements of the effectiveness of each mode for oxygen uptake, ie. convection requirement ($V_w/\dot{M}o_2$) and oxygen consumption ($\dot{M}o_2$), and the ventilatory work (W_s) and power requirements (W_r) of each mode.

Materials and Methods

Crabs of both sexes, weighing between 76 g and 385 g, were either caught locally, or obtained from commercial fishermen in Nelson, and flown to Christchurch. All animals were kept in a recirculating sea water system at 15°C, for at least 2 weeks before being used in experiments. During this acclimation period, crabs were fed twice weekly with fresh mussels (*Mytilus edulis* and *Perna canaliculus*). Ovigerous females and animals with missing appendages were not used. All crabs were considered to be in the intermoult stage of the moult cycle.

Series I: Oxygen Extraction and Consumption

Crabs of a narrow size range (319 - 361 g, $\overline{x}$ = 346 ± 3 g) were used in these experiments to eliminate the effects of size on oxygen consumption ($\dot{M}o_2$, μmol kg^{-1} min^{-1}). To monitor bilateral ventilation rate (F_r), small holes were drilled through the carapace ventrally and dorsally with respect to the positions of the scaphognathites. Platinum wire impedance electrodes were inserted through the holes and were fixed in place with cyanoacrylate cement and rubber dam. The electrodes were secured to the dorsal carapace with dam and connected to a two channel Bioscience A100 impedance coupler, the signal from which was displayed on a two channel BBC SE120 flatbed recorder. Crabs were fitted with masks made from polythene funnels. These were heat moulded and trimmed to reduce their volume and provide a snug fit around the eyes and mouthparts, without restricting the movement of these appendages. A gasket of thin rubber was glued to the mask using Ados F2 contact adhesive and this was then glued to the crab using cyanoacrylate cement, providing a water tight seal. A 4mm or an 8mm lumen diameter electromagnetic blood flow probe (In Vivometrics) was fitted in to the neck of mask to measure ventilatory water flow ($\dot{V}_W$). The probe was connected to either an EMI type SFMB1 electromagnetic flowmeter, or a Carolina Medical Electronics FM501 electromagnetic flowmeter, and the signal displayed on a Kipp & Zonen BD112 flatbed recorder. The probe was periodically calibrated, *in situ*, in both flow directions using a C.F. Palmer 1/8 hp motor, driving a Cole Palmer Masterflex pumphead. A device was made to allow the probe to be switched into line with either the crab, or the inflow or outflow from the pump, with minimal disturbance to the animal. Pressures generated within the mask during ventilation (P_{mask}) were always much less than 1 cm H_2O indicating that resistance to ventilatory flow resulting from masking was low.

During forward ventilation in buried and unburied animals, samples of exhalent water were drawn from the mask, via a 16g hypodermic needle and 1ml syringe, for analysis of expired oxygen tensions (P_eO_2). To determine the inhalent oxygen tension (P_iO_2) in unburied forward ventilating crabs, samples were taken from the surrounding water adjacent to the Milne-Edwards (M-E) apertures, at the bases of the chelipeds, which are the primary inhalent apertures (Chapter 2). When buried, the M-E apertures are still the main inhalent sites during forward ventilation. The inhalent water flow, via the exostegal channels (Chapter 2), produces a highly localised increase in the PO_2 of the interstitial water in the sediment. In order to obtain water samples that accurately reflected the P_iO_2 in buried crabs, the M-E apertures were cannulated. 20g needles were bent to follow the contours of the ventral carapace. One end of each needle was bent around so that it would be positioned just in the mouth of each aperture. The needles were connected to short lengths of polythene tubing (Dural, ID 0.86mm, OD 1.52mm) and fixed to the animals with strips of rubber dam and cyanoacrylate cement. A three-way tap was used to connect the cannulae for obtaining a mixed bilateral water sample. During reverse ventilation in both buried and unburied animals, a large proportion of the exhalent water flow passes out of the posterior branchial chamber openings situated between the 4th and 5th pereiopods (Chapter 2). In order to sample this water, holes were drilled through the dorsal carapace near the posterior margin overlying these openings. The underlying branchiostegite was cauterised with a hot needle and cannulae (Dural, ID 1.2mm, OD 1.7mm) were glued into the holes with cyanoacrylete cement. It could be seen through the posterior branchial apertures, that the tips of the cannulae projected 1 - 2 mm into the branchial chambers. This region is posterior to the position of the 9th gill. Therefore, water sampled at this point has passed over all the gills and presumably the extraction of oxygen is complete. The two cannulae were connected via a three-way tap through which samples were drawn with a 1 ml syringe. Each time a sample was drawn, the first 0.7 ml were discarded as this was the volume of the dead space of the cannulae and tap. It was assumed that water was drawn equally from both cannulae, and that the PO_2 of the sample was an average of the PO_2 in both branchial chambers. Inhalent water samples were obtained from reverse ventilating crabs, by sampling water as it entered the neck of the mask.

After cannulating the branchial chambers, fitting the impedance electrodes and mask, crabs were returned to the holding tanks and allowed to recover overnight. The following day each crab was transferred to a small sea water system with a total volume of 140l. The animal was placed in a 17l black plastic trough fitted with an overflow port on one side to maintain a constant water level. During experiments conducted on unburied crabs, the experimental chamber was lined with a coarse

plastic mesh. The animals would grip this, reducing spontaneous activity. For experiments requiring the animals to bury themselves, the chamber was filled with fresh beach sand to a depth of 90mm. This chamber was connected to large plastic barrel, containing 120 l of fresh aerated sea water (35% salinity). The barrel was placed in a refrigerator which kept the sea water at the experimental temperature of 15 ± 1°C. Water was constantly circulated through the system by a small submersible aquarium pump (Rena C40).

The presence of the mask appeared to be irritating to the animals. Continual efforts to rid themselves of the mask prolonged settlement for several days. During this time most animals showed a gradual decline in F_r and an increase in the proportion of time spent utilising the reverse ventilatory mode. This pattern is similar to that seen following exercise (Chapter 2). Throughout settlement P_iO_2, P_eO_2 and F_r were sampled randomly at a variety of ventilation frequencies from each crab. Animals were frequently stimulated by prodding or by being forced to swim to elicit high rates of F_r and $\dot{M}O_2$. Multiple samples were taken from unburied crabs during both forward and reverse ventilation. A different group of animals was used in the burial experiments with the same sampling regime. The PO_2 of the inhalent and exhalent water samples was measured using a Strathkelvin 1302 oxygen electrode thermostatted to 15°C and Strathkelvin 781 oxygen meter.

Ventilatory Work and Power

Two experiments were carried out to investigate the performance of the scaphognathite pump in *O. catharus*. In the first experiment (Series II) the work performed, and power generated, by the scaphognathites during spontaneous changes in ventilatory direction and ventilation frequency were evaluated. The second experiment (Series III) involved experimentally manipulating the total pressure (P_{total}) difference across the scaphognathites. Changes in the pressure against which the scaphognathites have to work are functionally identical to changes in the resistance to flow. This provides a model for examining the the ability of the scaphognathites to generate flow against various levels of resistance of the branchial pathway.

Unlike series I where bilaterally ventilating animals were used, in both series II and III experiments, the right branchial chamber was occluded by plugging the prebranchial aperture with dental impression wax and cyanoacrylate cement. This enabled direct correlation of $\dot{V}_w$ with F_r and calculation of scaphognathite stroke volume (V_s). F_r was monitored by impedance techniques and the animals were fitted

with plastic masks for measurement of $\dot{V}_w$, as above. Left branchial chamber pressure (P_{branch}) was monitored by cannulating the chamber ventrally with polythene tubing (Portex 800/100/320; ID 1.14mm, OD 1.57mm) (see chapter 2 for details). The water-filled cannula was connected to a Bell and Howell 4-327 blood pressure transducer. The signal from this was amplified using a Gould 13-4615-58 universal amplifier, and the output displayed and recorded on a BBC SE120 two channel flatbed recorder.

In series II, the animals were allowed to recover overnight before being placed in the experimental chamber. One group of crabs was used for sampling in the unburied state and another group were allowed to bury themselves. Simultaneous measurements of F_r, $\dot{V}_w$ and P_{branch} were made randomly over a range of F_r.

In series III, the total pressure difference across one scaphognathite pump ($P_{total} = P_{branch} - P_{mask}$) was manipulated. Pressure in the mask (P_{mask}) was measured via a water-filled polythene cannula connected to a second tranducer and amplifier. The animals were placed in the experimental chamber and the neck of the mask was connected to a rubber hose which passed through the wall of the experimental chamber. Externally the hose was connected to a small water reservoir which could be moved vertically to alter the pressure head against which the crab had to ventilate. The reservoir was separately supplied with sea water from the refrigerated barrel and was fitted with an overflow giving a constant water level. P_{mask} was altered randomly during both forward and reverse ventilation. All pressures were measured relative to the water surface in the experimental chamber.

Analysis of Data

All data are presented as means ± one standard error of the mean. All unpaired means were compared by one-way ANOVA. Significant differences between several means were identified using Duncan's new multiple range test ($\alpha = 0.05$). Model I regression equations were calculated using the least squares method and model II regressions were calculated using maximum likelihood estimation (Maximum likelihood program V 3.06, Rothamstead experimental station). All were tested for significance with *t*-tests ($p \leq 0.05$). All regression lines were then compared using ANCOVA techniques.

Calculations

1. Oxygen extraction efficiency (E_w%, %).

$$E_w\% = \frac{(P_{iO_2} - P_eO_2)}{P_iO_2} \times 100$$

where P_iO_2 is the inspired oxygen tension (torr) and P_eO_2 is the mixed expired oxygen tension (torr).

2. Oxygen consumption ($\dot{M}o_2$, μmol $kg^{-1}min^{-1}$).

$$\dot{M}O_2 = (P_iO_2 - P_eO_2) \times \dot{V}_w \times \alpha_wO_2$$

where $\dot{V}_w$ is the ventilation volume (ml $kg^{-1}min^{-1}$) and α_wO_2 is the oxygen solubility coefficient in water (μmol O_2 ml $H_2O^{-1}torr^{-1}$) at the appropriate temperature and salinity.

3. Stroke work (W_s, J $beat^{-1}kg^{-1}x10^4$).

$$W_s = (P_{branch} \times V_s) \times 10000$$

where $\dot{V}_s$ is the scaphognathite stroke volume (m^3 $beat^{-1}kg^{-1}$) and P_{branch} is the branchial chamber pressure (kPa).

4. Ventilatory power (W_r, mW kg^{-1})

$$W_r = (P_{branch} \times \dot{V}_w) \div 60 \times 1000$$

where $\dot{V}_w$ is the ventilation volume (m^3 $kg^{-1}min^{-1}$) and P_{branch} is the branchial chamber pressure (kPa).

Results

Series I: Oxygen Extraction and Consumption

Bilateral F_r (left + right) ranged from 50 to 360 beats min^{-1} in unburied forward ventilating crabs, and from 20 to 302 beats min^{-1} in unburied reverse ventilating animals. In buried animals, F_r varied between 74 and 174 beats min^{-1} during forward ventilation, and 30 and 187 beats min^{-1} in reverse. Despite the large overlap in F_r between the two modes, reverse ventilation tended to be utilised more at lower ventilation frequencies, while the opposite was true for the forward mode (see Chapter 2). The stroke volume of the scaphognathites (V_s, ml $beat^{-1}$ kg^{-1}) showed considerable inter- and intra-individual variability, and there was no signifcant relationship between V_s and F_r in any of the four treatment groups (Fig. 3.1). However, one unburied reverse ventilating animal showed a marked decline in V_s below about 60 beats min^{-1}. This can be seen in Fig. 3.1, with all values of V_s below 4.0 ml $beat^{-1}kg^{-1}$ recorded from this one crab.

In unburied crabs, the mean V_s was similar during forward and reverse ventilation (4.86 ± 0.14 ml $beat^{-1}$ kg^{-1} (n = 18) and 4.92 ± 0.15 ml $beat^{-1}$ kg^{-1}, (n = 15) respectively). Upon burial, V_s was significantly reduced. When utilising reverse ventilation the mean V_s in buried crabs was 4.11 ± 0.14 ml $beat^{-1}$ kg^{-1} (n = 18), but with forward ventilation V_s was significantly lower again at 3.63 ± 0.16 ml $beat^{-1}$ kg^{-1} (n = 13) (Duncan's new multiple range test, α = 0.05).

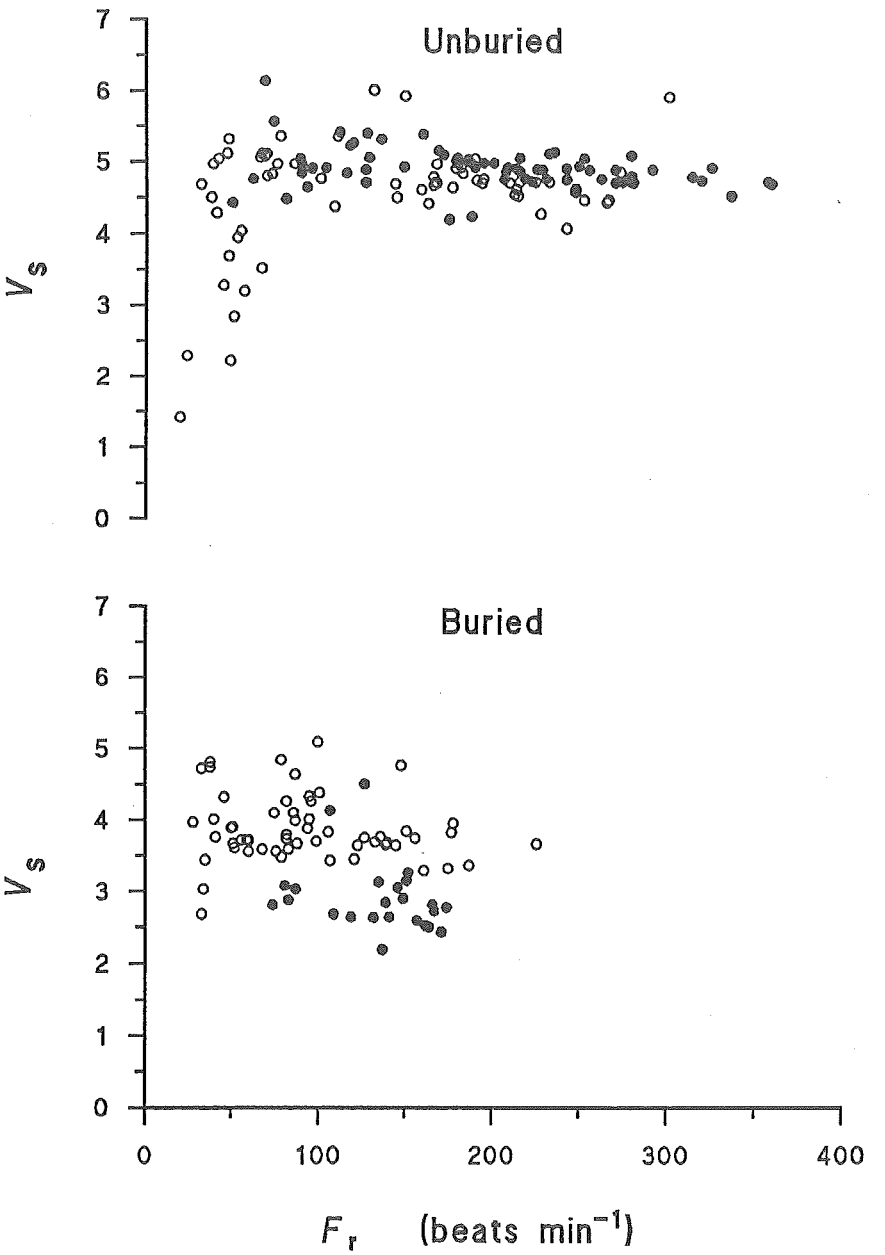

Fig. 3.1. V_s (ml beat^{-1}kg^{-1}) as a function of F_r in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. Values of *n* are as follows: unburied forward ventilating crabs, 7 animals, *n* = 75 points; unburied reverse ventilating crabs, 7 animals, *n* = 70 points; buried forward ventilating animals, 7 animals, *n* = 24 points; and reverse ventilating crabs, 14 animals, n = 58 points.

As a result of the relative constancy of V_s over a range of F_r within each treatment group, $\dot{V}_w$ was proportional to F_r (Fig. 3.2). The slopes of the regression lines calculated for the groups were all significantly different from one another. However, the intercepts of all four regression lines were close to zero confirming that the scaphognathite pumps maintain stroke volume even at very low values of F_r (ie. < 40 beats min^{-1}).

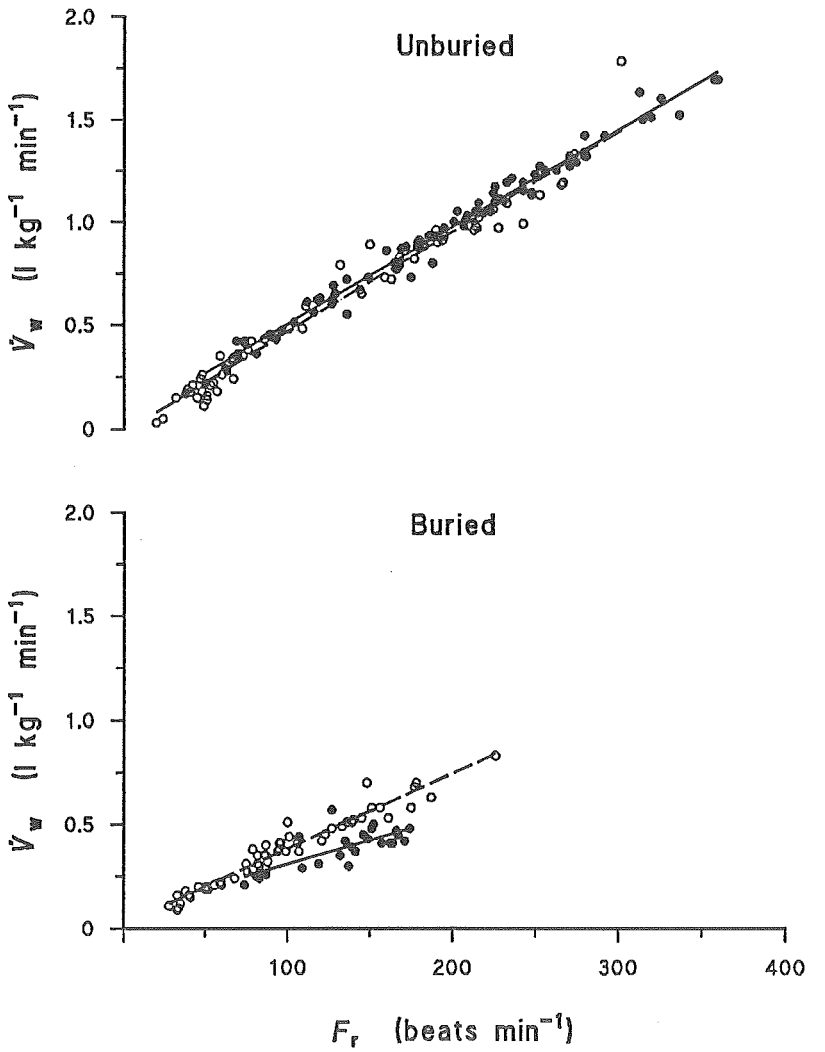

Fig. 3.2. $\dot{V}_w$ as a function of F_r in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. ———— indicates the least squares regression of the data for forward ventilating crabs, and ----- is the relationship for reverse ventilating animals. These were determined to be $\dot{V}_w = 0.005(F_r) + 0.032$, $r^2 = 0.984$ for unburied forward ventilating crabs, $\dot{V}_w = 0.005(F_r) - 0.019$, $r^2 = 0.966$ for unburied reverse ventilating crabs, $\dot{V}_w = 0.002(F_r) - 0.080$, $r^2 = 0.557$ for buried forward ventilating crabs, and $\dot{V}_w = 0.004(F_r) + 0.019$, $r^2 = 0.948$ for buried reverse ventilating crabs. All *n* values are the same as in Fig. 3.1.

Obtaining exhalent water samples for measurement of P_eO_2 in buried, reverse ventilating animals was more difficult than from the other three groups as significant exhalent water flow was observed from multiple branchial chamber apertures, (the M-E apertures and the large posterior openings between the 4th and 5th pereiopods, see Chapter 2). In addition, the proportions of total flow at these sites were not constant. Simultaneous unilateral M-E and posterior exhalent water samples were taken from 6 buried reverse ventilating animals to examine whether posterior aperture samples alone were representative of the overall P_eO_2 and E_w%. Samples were taken randomly with respect to $\dot{V}_w$, and regardless of the positions of any visible exhalent water plumes at the sediment surface (Chapter 2). The average E_w% from water exiting the M-E aperture was 48.2 ± 0.02% (n = 20), and the average E_w% from the posterior aperture was 47.8 ± 0.02 (n = 20). This difference was not significant (one-way ANOVA, F-test, $p > 0.05$). Therefore, the E_w% calculated from posterior exhalent water samples was used to as an estimate of the overall E_w% in buried, reverse ventilating animals.

The interstitial water in the sand was found to be relatively hypoxic. Immediately following burial, forward ventilating crabs showed very low values of P_iO_2. P_iO_2 increased over a matter of minutes, to values not dissimilar to those recorded from the above the sediment surface. The rate at which this increase occurred appeared to depend on the proportion of time spent utilising forward ventilation and the rate of ventilation. Recorded P_iO_2 in buried forward ventilating animals ranged from 78.9 torr to 133.2 torr, with a mean value of 113.0 ± 0.6 torr, n = 26.

$\dot{V}_w$ increased linearly with increasing $\dot{M}O_2$ in all four groups of crabs (Fig. 3.3). When animals were unburied, the slopes of the regression lines for $\dot{V}_w$ on $\dot{M}O_2$ were not significantly different during forward and reverse ventilation (F-test, $p > 0.05$). The intercept of the line calculated for forward ventilating animals was the greater of the two (F-test, $p < 0.05$). In buried animals the slopes of the two lines were also similar (F-test, $p > 0.05$), with the line describing the relationship in forward ventilating animals again having the greater constant (F-test, $p < 0.05$). When comparing buried and unburied animals, the slope of the regression of $\dot{V}_w$ on $\dot{M}O_2$ was significantly greater in unburied forward ventilating animals than in buried crabs utilising this mode (F-test, $p < 0.05$). This suggests that unburied animals increase $\dot{V}_w$ at a proportionally greater rate as $\dot{M}O_2$ increases. In addition, at a given $\dot{M}O_2$ unburied animals generally showed a higher $\dot{V}_w$ than buried crabs. Regression lines for buried and unburied reverse ventilating crabs showed a similar significant difference in the slopes (F-test, $p < 0.05$), and again unburied animals showed a larger $\dot{V}_w$ at a given $\dot{M}O_2$. The highest recorded $\dot{M}O_2$ was 122.9 μmol kg^{-1} min^{-1}, this was from an unburied crab utilising the forward mode, ventilating at 1.42 l kg^{-1} min^{-1}. The lowest $\dot{M}O_2$ was

from an unburied reverse ventilating animal at 4.8 μmol kg^{-1} min^{-1}. This corresponded to the lowest recorded $\dot{V}_w$ of 0.03 l kg^{-1} min^{-1}. In buried forward ventilating crabs, $\dot{M}o_2$ declined significantly with decreasing P_io_2 according to the relationship (y) = 0.310(x) + 0.414 (t = 0.178, $p < 0.05$)(Fig. 3.4).

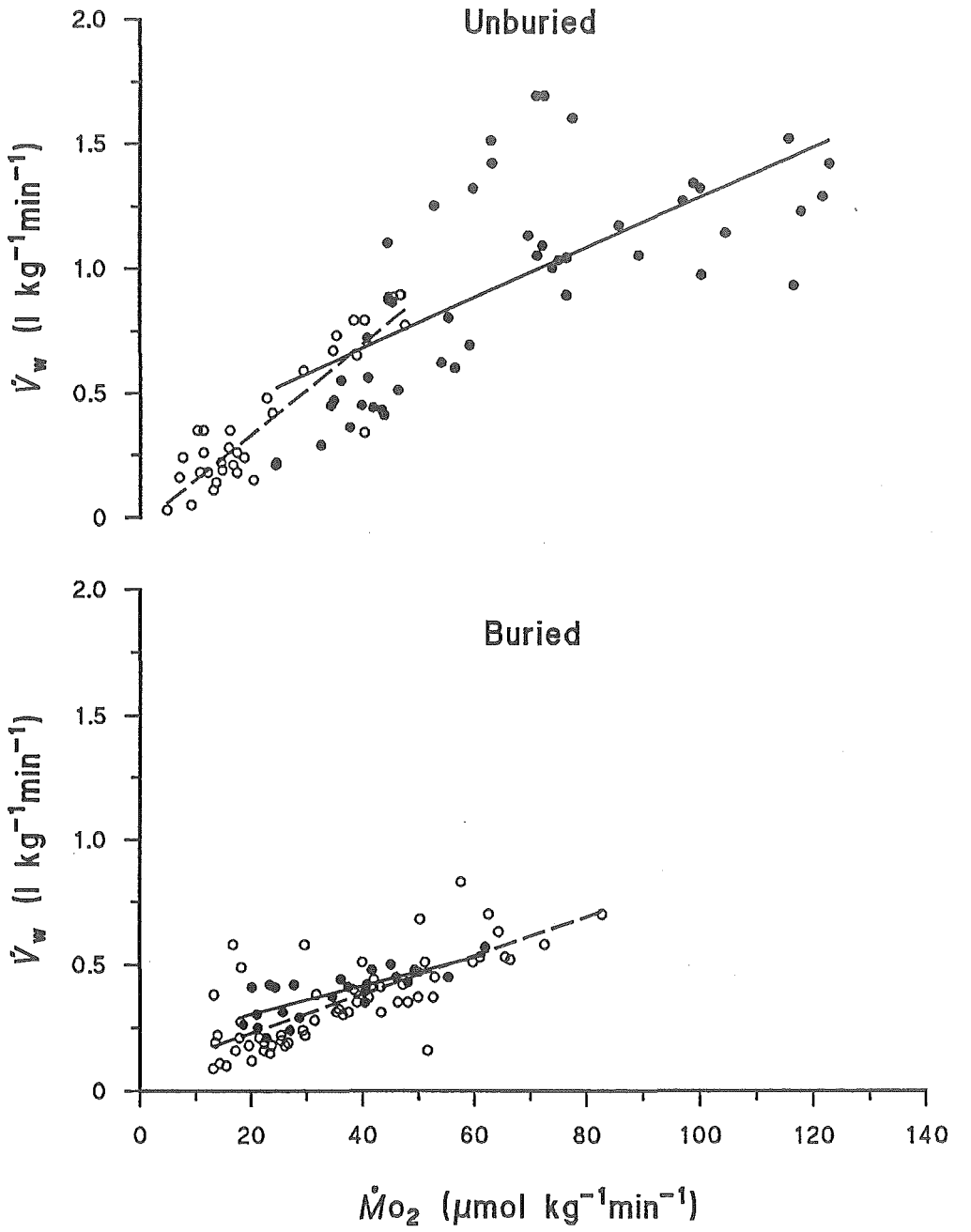

Fig. 3.3. $\dot{V}_w$ as a function of $\dot{M}o_2$ in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. ———— and ----- indicate the regressions of the data for forward and reverse ventilating crabs, respectively. These relationships were determined by maximum likelihood estimation to be ($\dot{V}_w$) = 0.0101($\dot{M}o_2$) + 0.2758, r^2 = 0.4716 (7 animals, n = 47 points) for unburied forward ventilating animals; ($\dot{V}_w$) = 0.0181($\dot{M}o_2$) - 0.0306, r^2 = 0.5820 (7 animals, n = 35 points) for unburied reverse ventilating animals; ($\dot{V}_w$) = 0.0057($\dot{M}o_2$) + 0.1877, r^2 = 0.8045 (7 animals, n = 25 points) for buried forward ventilating crabs; and ($\dot{V}_w$) = 0.0077($\dot{M}o_2$) + 0.0727, r^2 = 0.5432 (14 animals, n = 59 points) for buried reverse ventilating crabs.

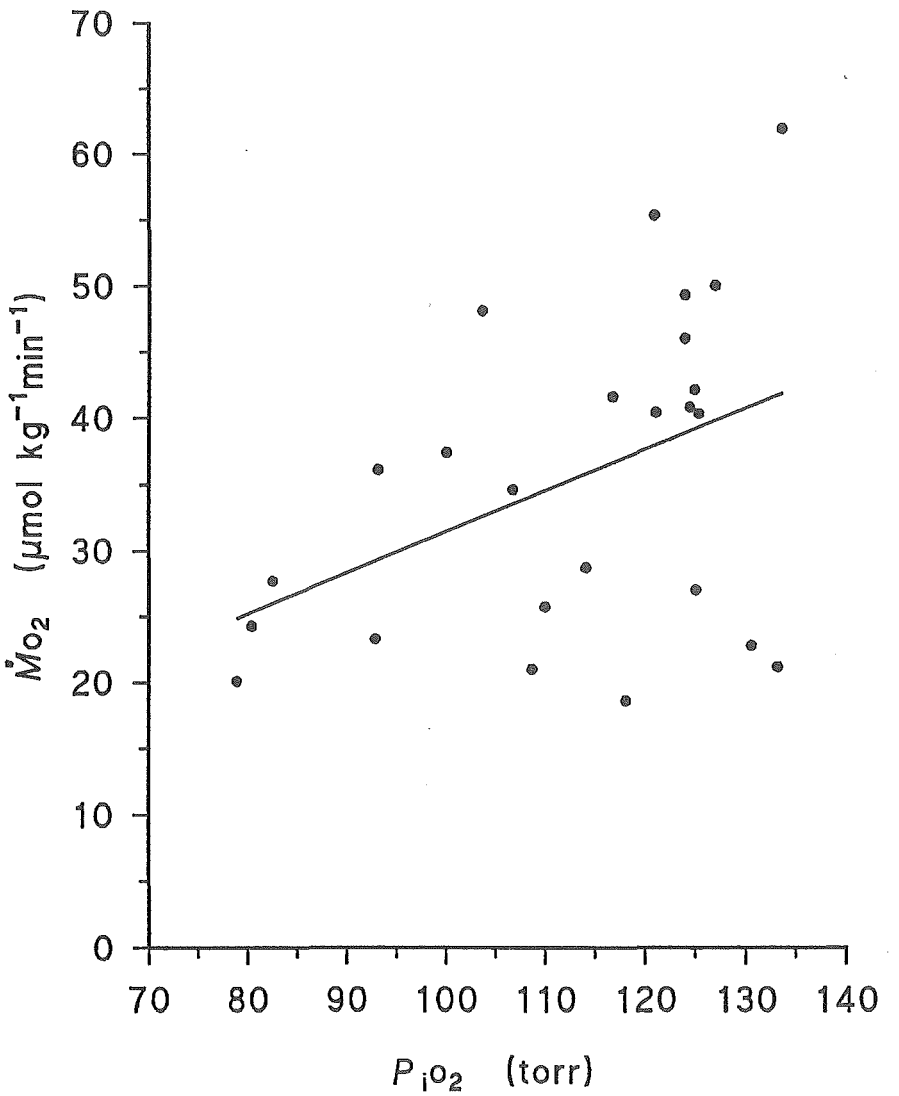

Fig. 3.4. $\dot{M}o_2$ as a function of P_iO_2 in buried forward ventilating *O. catharus*. The equation of the least squares regression line plotted for the relationship is $(\dot{M}o_2) = 0.310(P_iO_2) + 0.414$, $r^2 = 0.1776$. n = 25 points from 7 animals.

In unburied animals, oxygen extraction efficiency (E_w%) showed a general decline with increasing $\dot{V}_w$, in both ventilatory modes (Fig. 3.5). However, in buried animals, no decline in E_w% was obvious at higher values of $\dot{V}_w$. This may be partly due to the very small range of $\dot{V}_w$ over which recordings were made, and also to the observed variations in P_iO_2.

Mean values of E_w% within the range of $\dot{V}_w$ common to all four treatment groups (0.21 - 0.57 l $kg^{-1}min^{-1}$) were calculated. Mean E_w% in unburied forward ventilating crabs was 33.87 ± 1.79% at a mean $\dot{V}_w$ of 0.43 ± 0.03 l $kg^{-1}min^{-1}$ (n = 13). This was considerably higher than the mean E_w% of unburied reverse ventilating animals (23.78 ± 1.68%, 0.37 ± 0.02 l kg^{-1} min^{-1} (n = 14). It should be noted that the highest values of E_w% were, in fact, recorded from unburied reverse ventilating animals. However, these high values were outside the range of $\dot{V}_w$ shown by all four treatments and were therefore, excluded from calculations of the mean values of E_w% presented. The highest recorded E_w% of 68.9% was recorded from an unburied reverse ventilating animal. This value corresponded with the lowest recorded F_r (20 beats min^{-1}) and $\dot{V}_w$ (0.03 l kg^{-1} min^{-1}). The lowest E_w% 0f 12.0% was also shown by this group at a $\dot{V}_w$ of 0.35 l kg^{-1} min^{-1} and F_r of 69 beats min^{-1}.

In buried crabs, mean values of E_w% in forward and reverse ventilating were more similar, than in unburied animals. This was largely due to a general increase in values of E_w% recorded during reverse ventilation. On average, E_w% was also higher in buried crabs than in unburied crabs. Mean values of E_w% recorded from buried crabs utilising forward and reverse ventilation were 49.53 ± 2.03% ($\dot{V}_w$ = 0.39 ± 0.02 l $kg^{-1}min^{-1}$, n = 25) and 46.33 ± 1.67% ($\dot{V}_w$ = 0.30 ± 0.025 l kg^{-1} min^{-1}, n = 37), respectively. E_w% in buried crabs utilising forward ventilation was not correlated with P_iO_2 (least squares regression, t = 0.7439, $p > 0.05$)

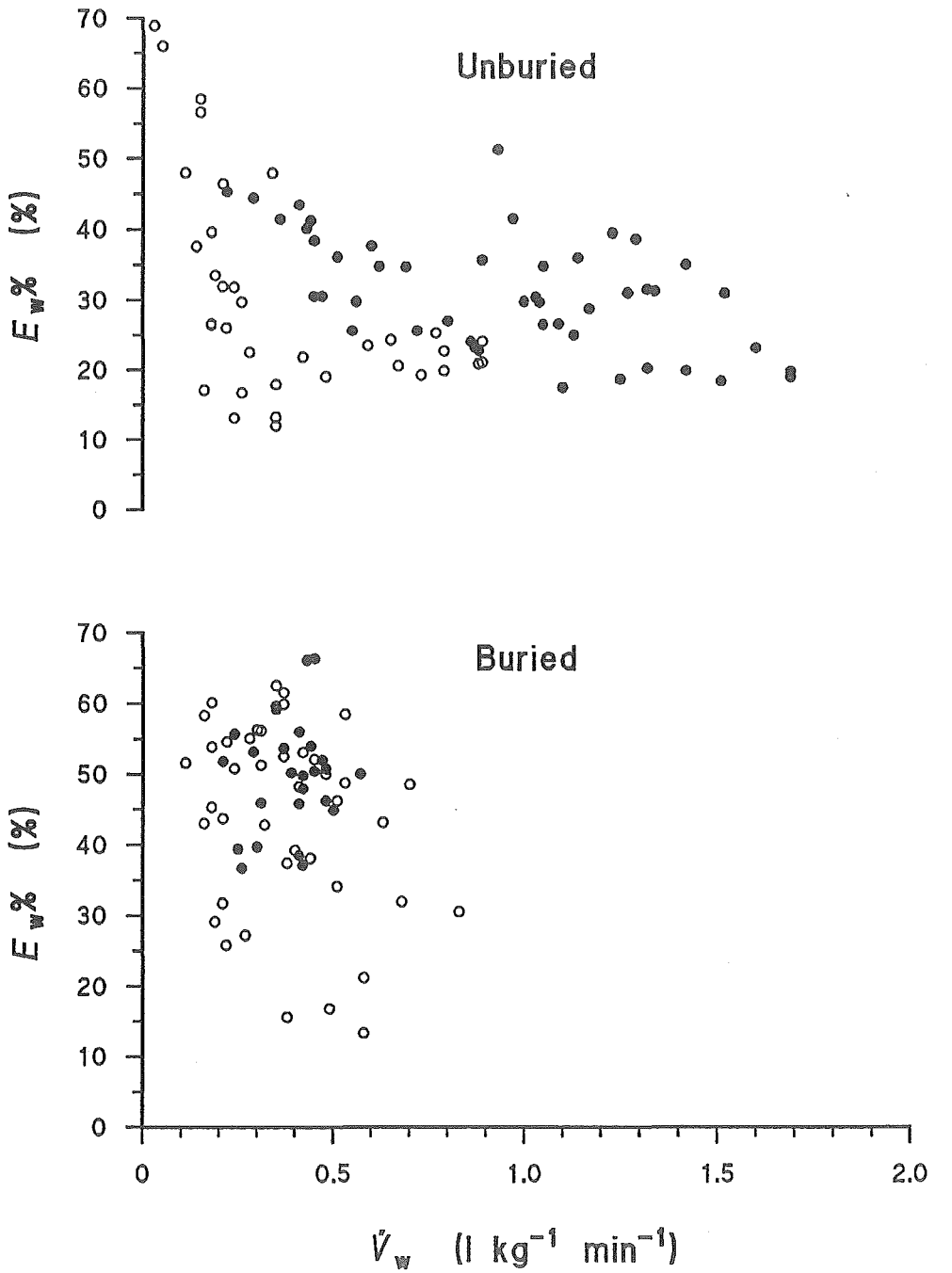

Fig. 3.5. Oxygen extraction efficiency (E_w%) as a function of $\dot{V}_w$ in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. All *n* values are the same as in Fig. 3.3.

An alternative index for assessing the relative effectiveness of each of the modes for oxygen uptake, is the convection requirement ($\dot{V}_w/\dot{M}o_2$, ml μmol^{-1}). This is the amount of water an animal is required to ventilate to obtain a given amount of oxygen. Both forward and reverse ventilating unburied crabs, and buried reverse ventilating animals, showed a general increase in $\dot{V}_w/\dot{M}o_2$ with increasing $\dot{V}_w$ (Fig. 3.6). No relationship was clear in forward ventilating buried animals, but again the narrow range of $\dot{V}_w$ recorded for this group, and fluctuating P_io_2 may contribute to this.

Mean $\dot{V}_w/\dot{M}o_2$ was calculated over the range of $\dot{V}_w$ shared by all 4 treatments (0.21 - 0.57 l kg^{-1} min^{-1}). Both forward and reverse ventilating buried crabs, and forward ventilating unburied crabs showed similar mean values of $\dot{V}_w/\dot{M}o_2$ at 11.6 $\pm$ 1.0 ml μmol^{-1} (n = 25), 10.3 $\pm$ 0.7 ml μmol^{-1} (n = 37), and 10.9 $\pm$ 1.5 ml μmol^{-1} (n = 13), respectively. While unburied crabs utilising the reverse mode had a much higher mean $\dot{V}_w/\dot{M}o_2$ of 19.0 $\pm$ 1.3 ml μmol^{-1}, (n = 14). In buried forward ventilating crabs, $\dot{V}_w/\dot{M}o_2$ increased linearly with decreasing P_io_2 (Fig. 3.7). This relationship is described by the equation $(\dot{V}_w/\dot{M}o_2) = -0.1469(P_io_2) + 28.2072$ ($r^2 = 0.601$, $t = -5.8799$, $p < 0.05$).

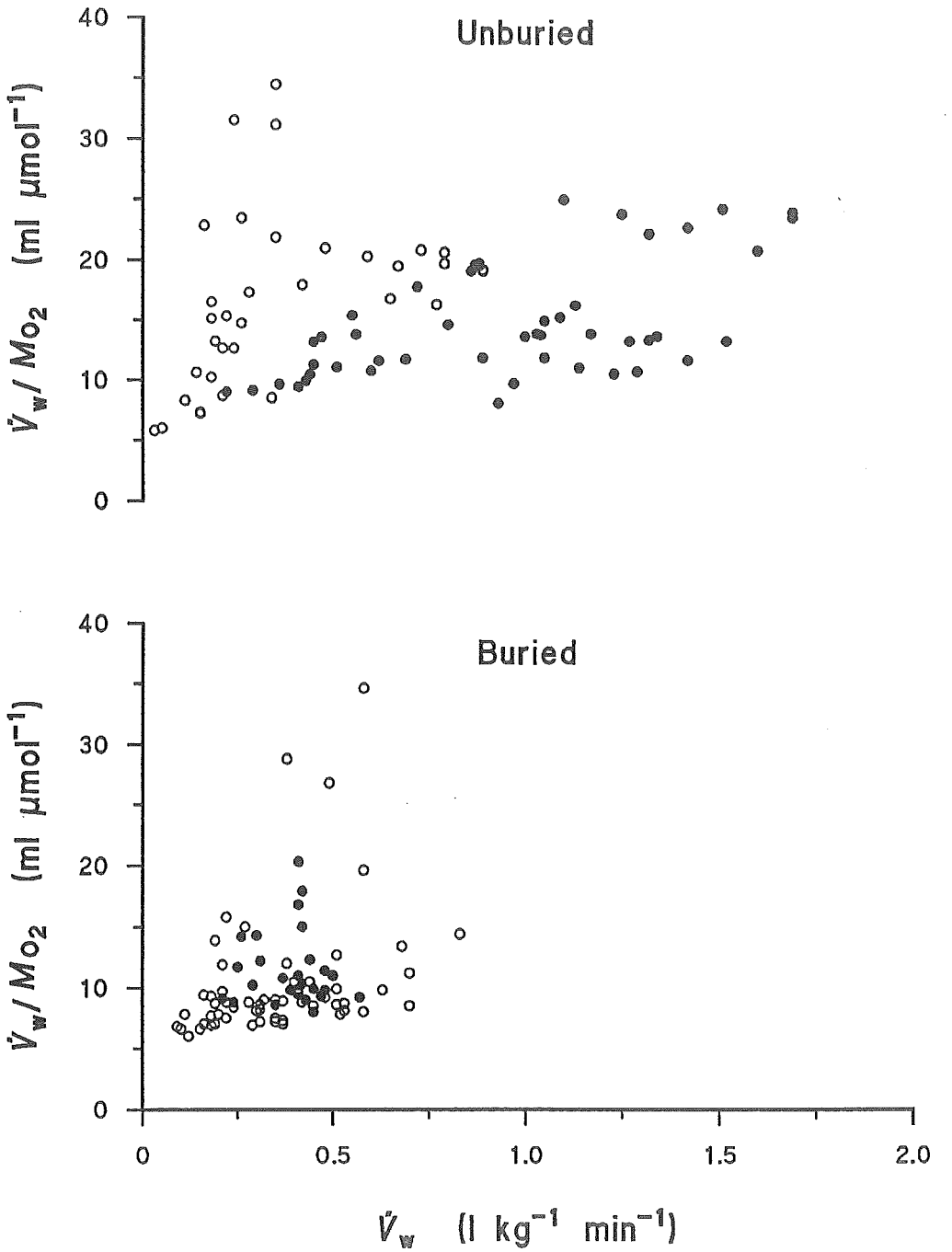

Fig. 3.6. Convection requirement ($\dot{V}_w/\dot{M}o_2$) as a function of $\dot{V}_w$ in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. All *n* values are the same as in Fig. 3.3.

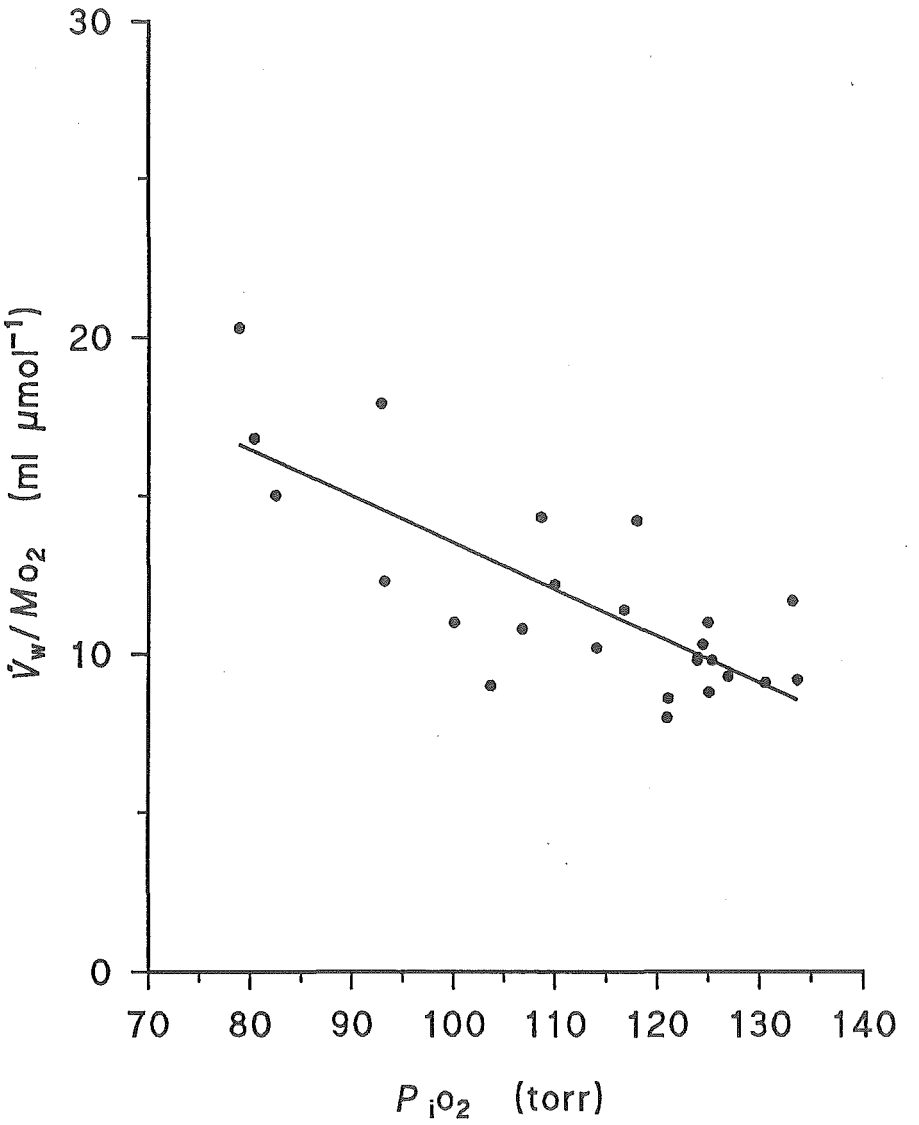

Fig. 3.7. $\dot{V}_w/\dot{M}o_2$ as a function of P_iO_2 in buried forward ventilating *O. catharus*. The equation of the least squares regression line plotted is $(\dot{V}_w/\dot{M}o_2) = -0.1469(P_iO_2) + 28.2072$, $r^2 = 0.601$. $n = 25$ points from 7 animals.

So far comparisons have been made between the four treatment groups with respect to a given value of another variable, such as the comparison of values of $\dot{V}_w$ a given value of $\dot{M}o_2$, and the comparison of values of $E_w\%$ and $\dot{V}_w/\dot{M}o_2$ within a given range of $\dot{V}_w$. This is reinforced visually in the presentation of the accompanying figures. Although this assessment is not invalid, the system under examination is dynamic, with $\dot{V}_w$, $E_w\%$, and $\dot{M}o_2$ all constantly changing, depending on the internal physiological state of the animal. To examine these spontaneous changes, adjacent periods of forward and reverse ventilation from animals used in the Series I experiments were analysed (Table 3.1).

In unburied animals, mean F_r was lower during a sustained ventilatory reversal than during an adjacent period of forward ventilation. Accordingly, $\dot{V}_w$ was also lower when ventilation was reversed. $E_w\%$ was significantly diminished during periods of reverse ventilation ($p < 0.05$). As a result of the reduced values of $\dot{V}_w$ and $E_w\%$ in this group, $\dot{M}o_2$ was, on average, halved at the onset of reverse ventilation. Mean $\dot{V}_w/\dot{M}o_2$ was 24% higher during periods of reverse ventilation, representing a significant increase over the mean value calculated during forward ventilation ($p < 0.05$).

In buried animals, mean F_r was also lower during sustained ventilatory reversals and $\dot{V}_W$ was reduced by a similar proportion to that seen in unburied crabs (approx. 30%). However, with the onset of reverse ventilation, $\dot{M}o_2$ was only reduced by about 30%, rather than the 50% seen in unburied crabs. This was due to similar values of $E_w\%$ in either mode ($p > 0.05$). Thus $\dot{M}o_2$ varied directly with $\dot{V}_w$. Likewise, $\dot{V}_w/\dot{M}o_2$ was not significantly different during adjacent periods of forward and reverse ventilation when buried ($p > 0.05$).

Table 3.1. Mean values (± s.e.m.) of F_r (beats min^{-1}), $\dot{V}_w$ (l $kg^{-1}min^{-1}$), E_w% (%), $\dot{M}o_2$ (µmol $kg^{-1}min^{-1}$), and $\dot{V}_w/\dot{M}o_2$ (ml μmol^{-1}) during adjacent periods of forward and reverse ventilation in unburied and buried *O. catharus*. * indicates a significant difference ($p < 0.05$); paired *t*-test.

Unburied Crabs (n = 6)					
	F_r	$\dot{V}_w$	E_w%	$\dot{M}o_2$	$\dot{V}_w/\dot{M}o_2$
Forward ventilation	146.8 ± 18.4	0.74 ± 0.10	32.9 ± 4.1*	54.4 ± 5.4	139 ± 19*
Reverse ventilation	99.0 ± 23.4	0.52 ± 0.12	23.7 ± 1.8	27.1 ± 5.4	184 ± 17
Buried Crabs (n = 4)					
	F_r	$\dot{V}_w$	E_w%	$\dot{M}o_2$	$\dot{V}_w/\dot{M}o_2$
Forward ventilation	148.5 ± 8.2	0.41 ± 0.06	53.3 ± 4.6	42.7 ± 10.6	105 ± 14
Reverse ventilation	89.3 ± 29.6	0.28 ± 0.05	49.4 ± 2.9	30.5 ± 6.4	9.7 ± 0.5

Ventilatory Work and Power

Series II

In unburied animals, F_r (left side only) ranged from 32 to 207 beats minute^{-1} in forward mode, and 38 to 182 beats minute^{-1} in reverse. Reverse F_r in buried crabs ranged from 25 to 123 beats minute^{-1} and forward F_r from 42 to 136 beats minute^{-1}. As unilateral branchial chamber occlusion did not affect V_s (Paired t-test, $t = 0.2892$, $p > 0.05$, $n = 6$), the mean V_s and the relationship of $\dot{V}_w$ on F_r in each mode was as described for bilaterally ventilating crabs above.

All groups showed a linear increase in branchial chamber pressure with increasing $\dot{V}_w$ (Fig. 3.8). Large intra- and inter-individual variability in P_{branch}, was recorded from crabs in all groups. Many individuals showed short term changes in P_{branch}, over a matter of minutes (Fig. 3.9), and long term changes over successive days (Fig. 3.10). These occurred without concomitant changes in ventilatory flow indicating that the crabs have an ability to alter the resistance to flow presented by the branchial water pathway. Despite this variability, significant regressions of P_{branch} on $\dot{V}_w$ were calculated for all groups. Unburied crabs showed similar values of P_{branch} in either mode. Forward P_{branch} varied between -0.30 and -4.60 cm H_2O, and reverse P_{branch} between 0.45 and 3.64 cm H_2O. Ignoring the signs, the slopes and constants of the regressions of P_{branch} on $\dot{V}_W$ during forward and reverse ventilation were similar (F-test, $p > 0.05$). Upon burial in sand P_{branch} was seen to increase. Reverse ventilating crabs showed greater P_{branch}s, at any given $\dot{V}_W$, than unburied animals, with a range of 1.85 to 4.16 cm H_2O. The slope of the regression line of P_{branch} on $\dot{V}_w$ from this group was significantly greater than in unburied crabs (F-test, $p < 0.05$). Buried forward ventilating crabs showed an even greater increase in P_{branch} (-4.45 to -9.69 cm H_2O), with the slope of the regression line significantly greater than all three other groups (F-test, $p < 0.05$).

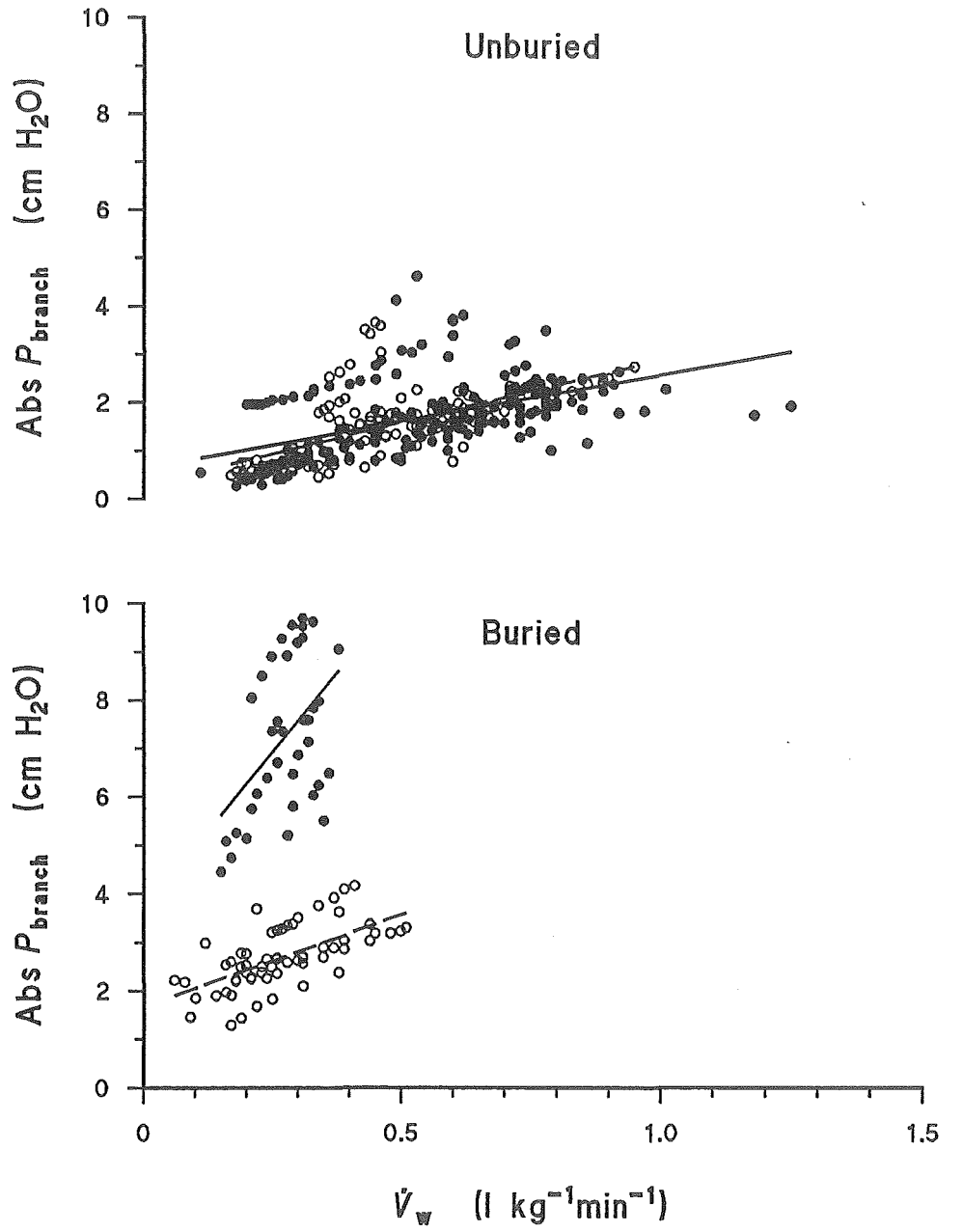

Fig. 3.8. (A) Absolute value of P_{branch} as a function of ventilation volume ($\dot{V}_w$) in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. ———— and ----- indicate the regressions of the data for forward and reverse ventilating crabs, respectively, as determined by maximum likelihood estimation. These were as follows: $(P_{branch}) = 1.908(\dot{V}_w) + 0.635$, $r^2 = 0.278$ for unburied forward ventilating animals (11 animals, $n = 194$ points); $(P_{branch}) = 2.566(\dot{V}_w) + 0.286$, $r^2 = 0.396$ for unburied reverse ventilating animals (9 animals, $n = 115$); $(P_{branch}) = 3.779(\dot{V}_w) + 1.673$, $r^2 = 0.415$ for buried forward ventilating animals (7 animals, $n = 37$ points); and $(P_{branch}) = 13.082(\dot{V}_w) + 3.641$, $r^2 = 0.231$ for buried reverse ventilating crabs (8 animals, $n = 61$ points).

Fig. 3.9. A recording of branchial chamber pressure (P_{branch}; cm H_2O), impedance trace of scaphognathite activity (F_r; beats min^{-1}) and ventilation volume ($\dot{V}_w$; ml $beat^{-1}kg^{-1}$) from a buried reverse ventilating crab showing spontaneous short term changes in P_{branch}. An increase in P_{branch} accompanies periods where the Milne-Edwards apertures are the primary exhalent openings (ME). During periods when the posterior branchial apertures between the 4th and 5th pereiopods are the main exhalent sites (Post.) P_{branch} is lower. There is little variation in F_r and $\dot{V}_w$, therefore the changes in P_{branch} must indicate changes in the resistance of the branchial water pathway.

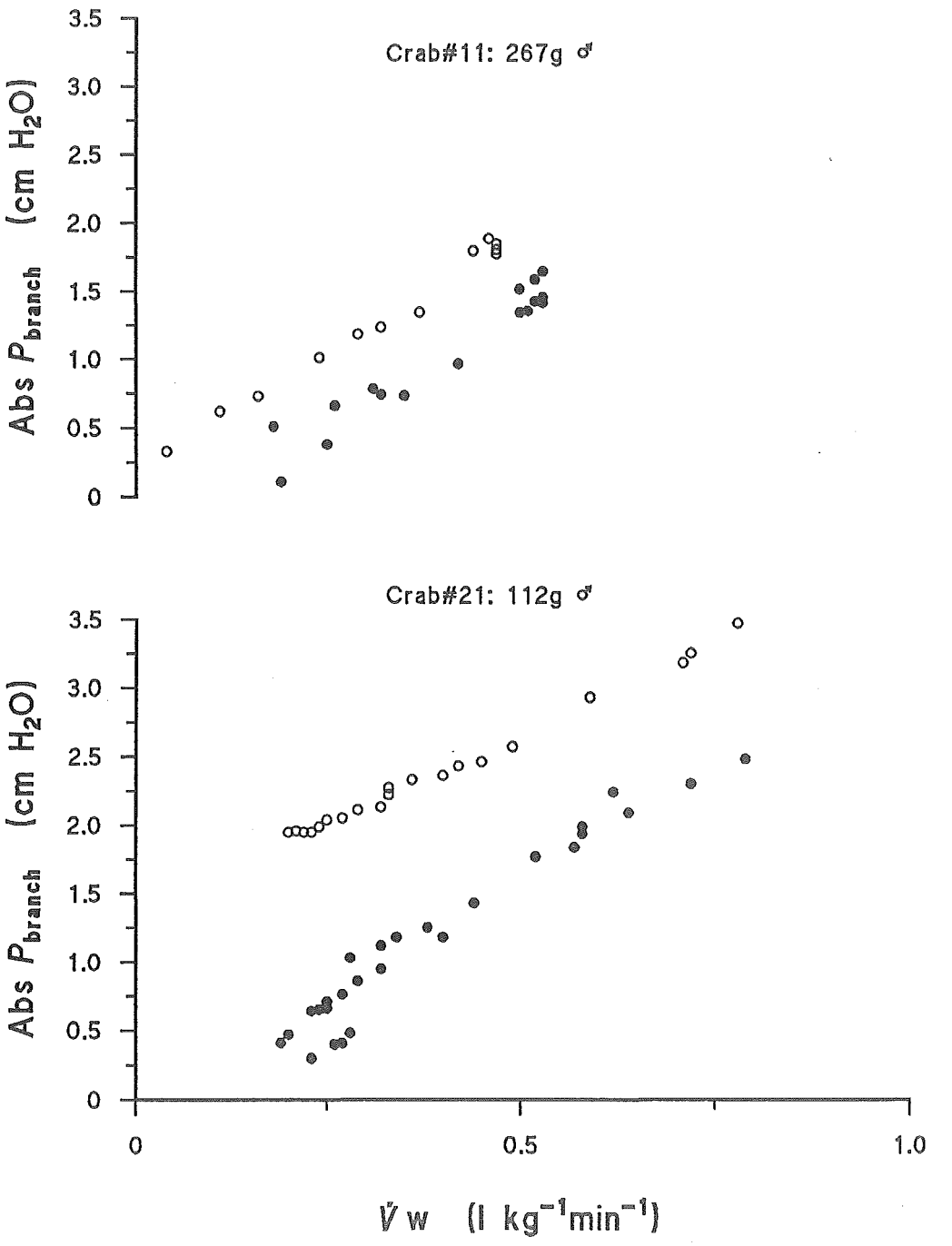

Fig. 3.10. Two examples from unburied forward ventilating of crabs that showed spontaneous changes in branchial chamber pressure (P_{branch}) over successive days of recording; day 1 (●) and day 2 (○). This indicates that a change has occurred in the flow resistance of the branchial water flow pathway.

The stroke work (W_s) performed by the scaphognathites in generating ventilatory flow increased linearly with increasing F_r (Fig. 3.11). As W_s is calculated from V_s (see methods section, equation 3) and P_{branch}, the variability seen in the two latter parameters contributed to a high degree of variability in W_s. W_s was similar during forward and reverse ventilation in unburied crabs. Upon burial, W_s increased. Relative to unburied crabs, W_s was greater over the entire observed range of F_r in buried reverse ventilating crabs. W_s was much higher in forward ventilating buried crabs than in all other groups, and increased with increasing F_r at a much greater rate than in all other groups.

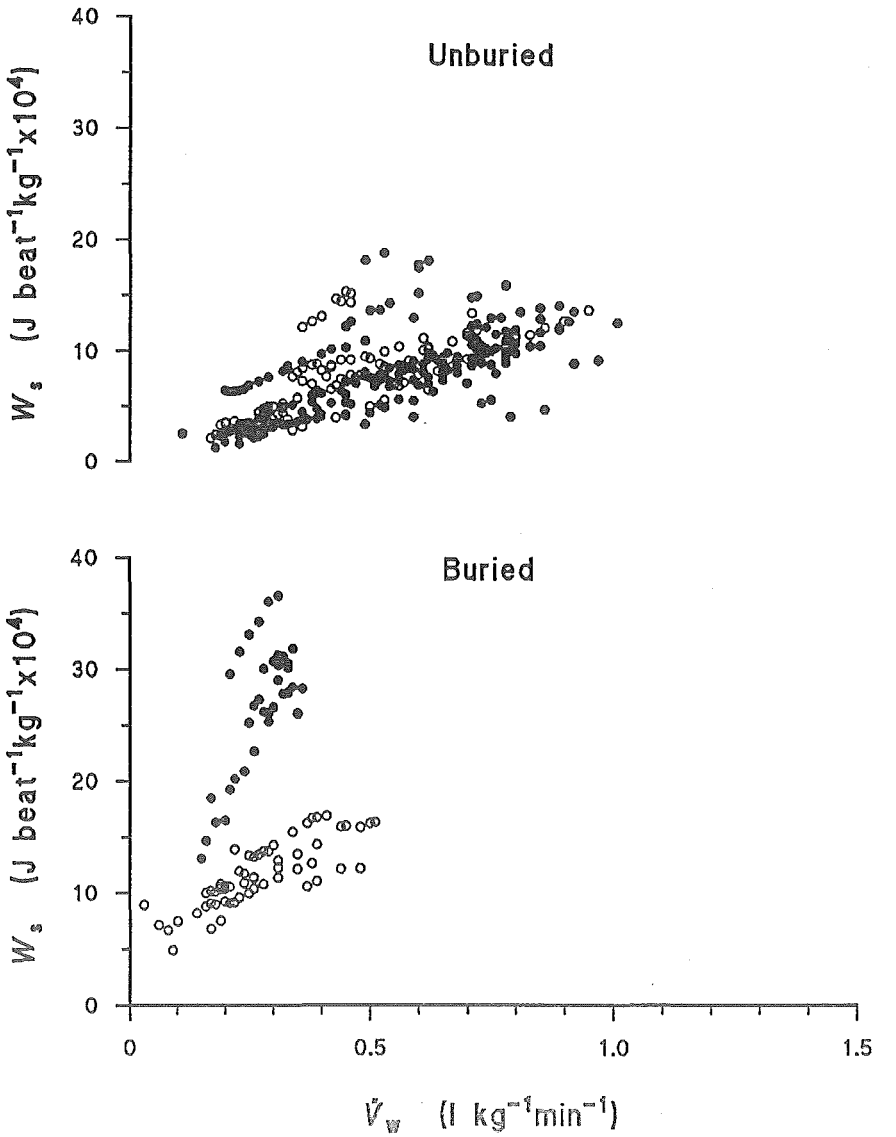

Fig. 3.11. Scaphognathite stroke work (W_s) as a function of $\dot{V}_w$ in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. All *n* values are the same as in Fig. 3.8.

The power of ventilation increased in a curvilinear fashion with increasing $\dot{V}_w$ (Fig. 3.12). As with W_s, unburied crabs showed a similar W_r at any given $\dot{V}_w$. The range of $\dot{V}_w$ from 0.15 to 0.38 l $kg^{-1}min^{-1}$ was common to all four treatment groups. Within this range mean W_r was 0.471 ± 0.042 mW kg^{-1} (n = 56) and 0.470 ± 0.052 mW kg^{-1} (n = 48), in unburied forward and reverse ventilating animals, respectively. Following burial in sand, mean W_r over this same range of $\dot{V}_W$ more than doubled to when ventilating (1.121 ± 0.069 mW kg^{-1}, n = 46), and was more than seven times higher when forward ventilating (3.341 ± 0.189 mW kg^{-1}, n = 37).

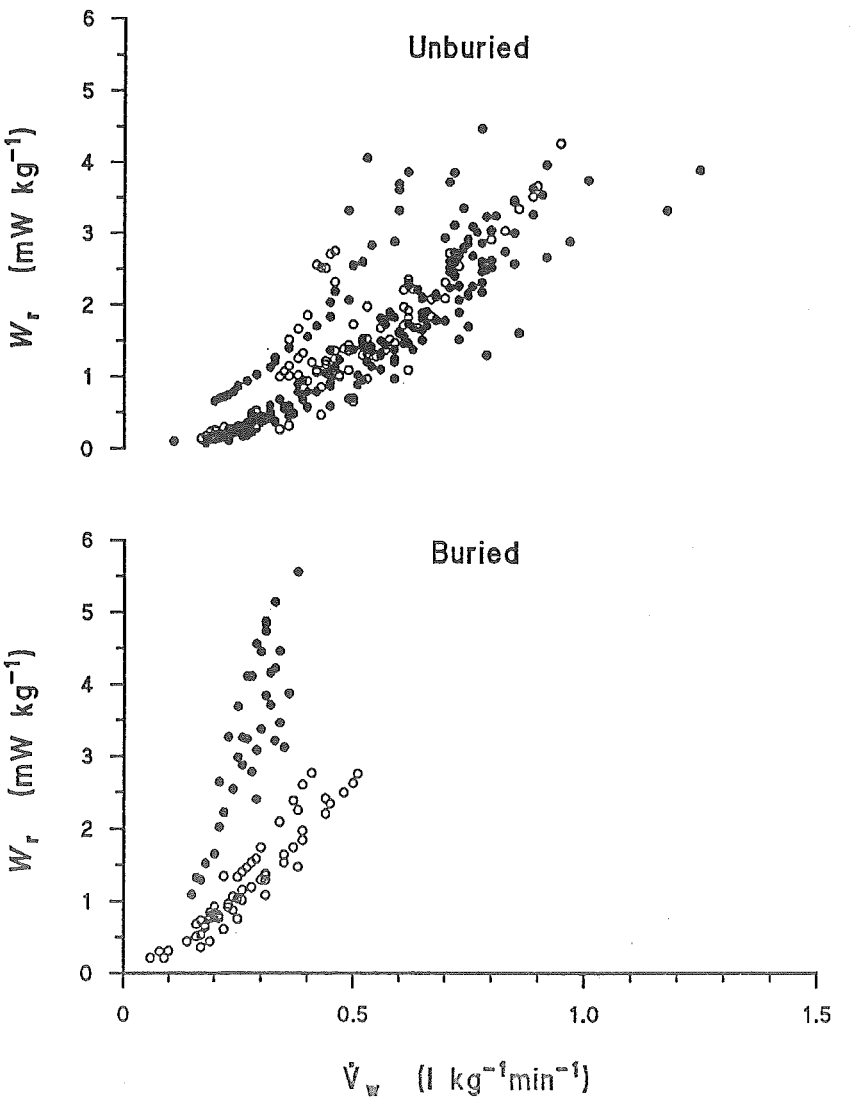

Fig. 3.12. Ventilatory power (W_r)as a function of $\dot{V}_w$ in buried and unburied forward (●) and reverse (○) ventilating *O. catharus*. All n values are the same as in Fig. 3.8.

Series III

By experimentally manipulating the total pressure difference across the scaphognathites (P_{total}) in unburied crabs, it was possible to simulate the effect of varying branchial chamber resistances on the performance of the scaphognathite pump. For the ease of discussion I will use the mean V_s between 0-1 cm H_2O P_{total}, in either mode, as the reference against which all changes shall be compared.

In general, increasing the P_{total} resulted in a decrease in V_s (Fig. 3.13 & 3.14). In the reverse ventilatory mode, V_s was relatively constant until a P_{total} of of about 3 cm H_2O was reached. After this point V_s began to decline significantly (Duncan's new multiple range test $\alpha = 0.05$). Mean V_s was also relatively constant from 2 - 5 cm H_2O, but then showed a large drop at values of P_{total} greater than 5cm H_2O. Forward ventilating crabs showed a steadier decline in V_s from 0 - 5 cm H_2O, but again no significant change occurred until P_{total} reached 3 - 4cm H_2O (Duncan's new multiple range test $\alpha = 0.05$). As with reverse ventilation, a sudden drop in V_s was seen at a P_{total} greater than 5 cm H_2O. No compensatory increase in F_r was apparent in either mode, and as a result, $\dot{V}_w$ also declined with increasing P_{total}. No relationship between size and ablility to maintain flow against a given P_{total} was apparent. These results confirm that the small additional pressures introduced by masking (< 1cm H_2O) would have a negligible effect on V_s in either mode.

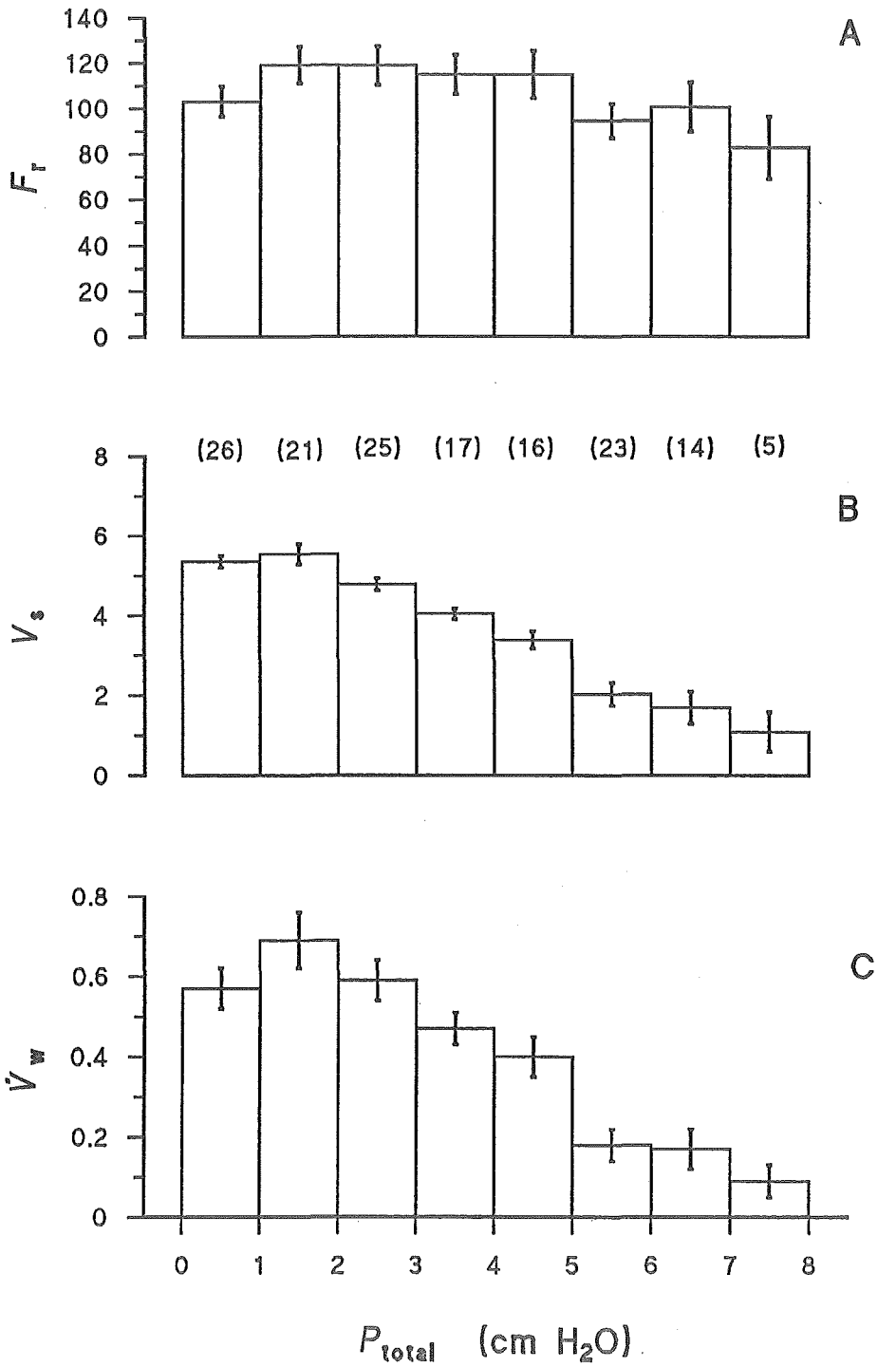

Fig. 3.13. Bargraph of A) changes in mean ventilation frequency (F_r, beats min^{-1}), B) mean scaphognathite stroke volume (V_s, ml $beat^{-1}kg^{-1}$) and C) mean ventilation volume ($\dot{V}_w$, 1 kg^{-1}), with increasing P_{total} during forward ventilation. The numbers in brackets indicate n for each bar, and each bar shows the mean ± sem.

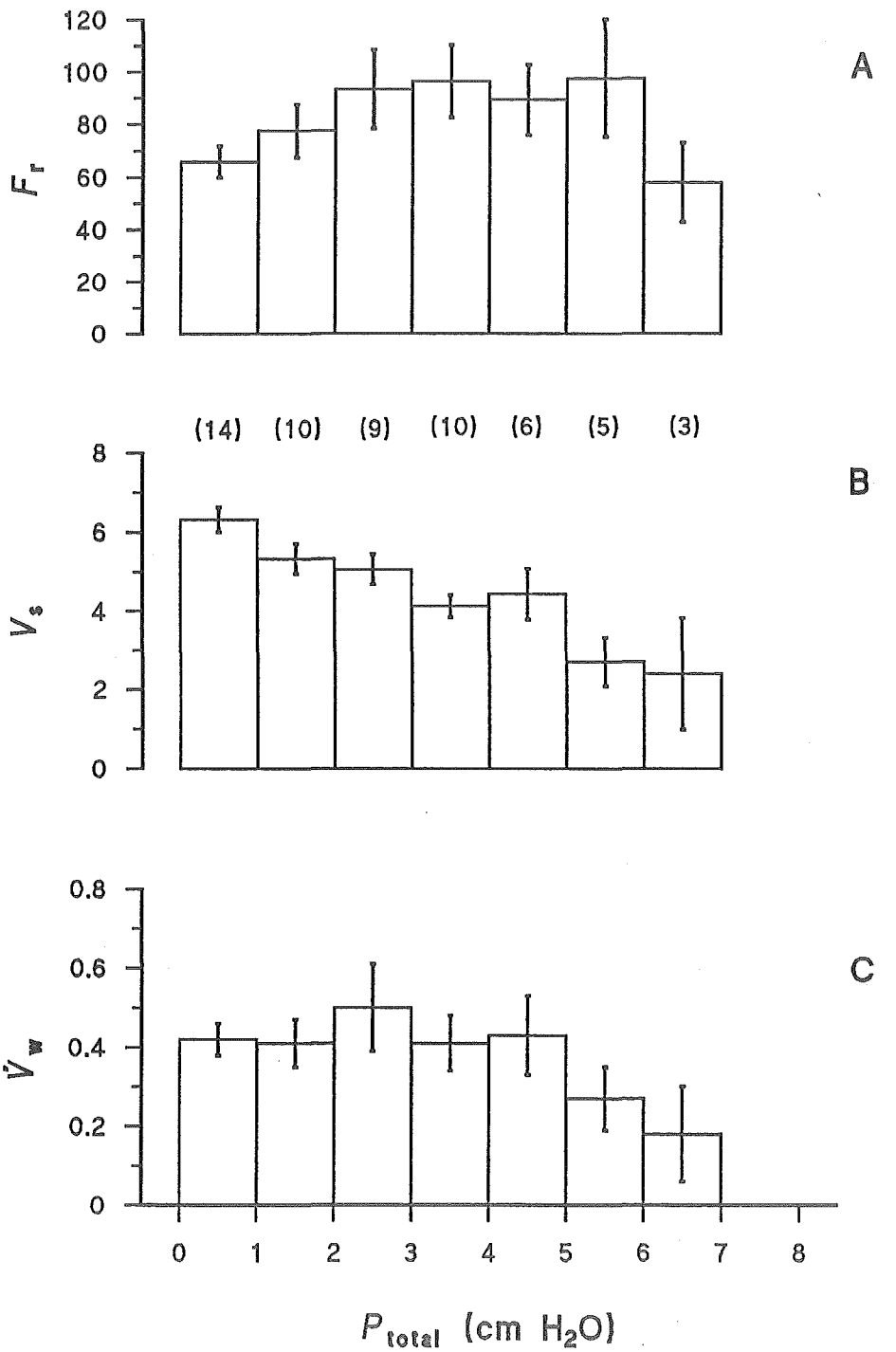

Fig. 3.14. Bargraph of A) changes in mean ventilation frequency (F_r, beats min^{-1}), B) mean scaphognathite stroke volume (V_s, ml $beat^{-1}kg^{-1}$) and C) mean ventilation volume ($\dot{V}_w$, 1 kg^{-1}), with increasing P_{total} during reverse ventilation. The numbers in brackets indicate n for each bar, and each bar shows the mean ± sem.

Discussion

The Uptake of Oxygen

Previous studies suggest that, by reversing the direction of water flow, buried crabs are able to maintain respiratory function by utilising water from above the sediment surface for ventilation (Garstang, 1896, 1897a, Arudpragasam and Naylor, 1966; Hartnoll, 1972; Caine, 1974; Taylor, 1984, McLay and Osborne, 1985; Barshaw and Able, 1990). Caine (1974) supposed that ventilating in the forward direction would require buried individuals of *Ovalipes guadulpensis* to irrigate the gills with interstitial water, which was assumed to be silt-laden and relatively hypoxic, thus reducing the availability of oxygen. However, the present study has shown that buried individuals of *O. catharus* will ventilate in both directions, despite relatively hypoxic interstitial oxygen tensions (as low as 50% of ambient Po_2). Furthermore, values of $\dot{M}o_2$ recorded from buried forward ventilating crabs were not appreciably different to those recorded from buried animals utilising reverse ventilation. Buried *O. catharus* form exostegal channels between the flexed chelipeds and the carapace (Chapter 2). These allow a clear pathway for water flow from the sediment surface to the M-E apertures, which are the primary inhalent apertures during forward ventilation. Thus, interstitial water is not utilised for ventilation, and a high P_io_2 is maintained. Individuals of the specialist burrowing species *Atelecyclus rotundatus* forward ventilate when they are unburied (Taylor, 1984). When partially buried this mode still predominates as the exostegal channels are able to function. However, when fully buried, a reverse pattern of ventilatory flow is established using an inhalent breathing tube formed by the setose antennae to connect to the sediment surface. In this state the exostegal channels are far removed from the sediment surface and are unable to function. Thus in species which utilise the exostegal channels, reverse ventilation is not essential for maintenance of a respiratory water stream. The settled position of fully buried *O. guadulpensis* described by Caine (1974) is identical to that shown by *O. catharus* (Chapter 2) suggesting that the former may also form exostegal channels. The formation of exostegal channels was first observed in the portunid *Portumnus nasutus* (Garstang, 1897b) and may be a common feature of all burrowing portunid species.

Hughes et al. (1969) showed that, in the shore crab *Carcinus maenas*, the countercurrent water and haemolymph flows that occur across the gill sieve during forward ventilation are disrupted during a ventilatory reversal. These authors predicted that this would result in a reduction in E_w% during reversals. This was confirmed in a subsequent study, using unburied *Cancer magister* (McDonald et al., 1980). At the onset of sustained reverse ventilation, E_w% declined by approximately 30%. The net result of this was to increase the convection requirement ($\dot{V}_w/\dot{M}o_2$) by 50%. Thus a greater volume of water must be pumped per unit of oxygen consumed, increasing the work of ventilation. Branchial water flow patterns observed in *O. catharus* during forward ventilation were also disrupted when ventilation was reversed (Chapter 2). As with *C. magister*, unburied *O. catharus* showed a reduction in E_w% and an increase in $\dot{V}_w/\dot{M}o_2$ when switching from the forward to the reverse mode. However, in buried specimens of *O. catharus*, an improvement in E_w% and $\dot{V}_w/\dot{M}o_2$ during reverse ventilation, compared with unburied crabs, resulted in the two parameters being similar in the either mode. This may be due to better irrigation of the gills when buried, resulting from adduction of the carapace to the thorax (Chapter 2). The roof of the branchial chamber may be brought closer to the dorsal lamellae, limiting the strong epibranchial flow seen in unburied crabs, which seems to bypass most of the gills during reverse ventilation. Utilisation of alternative sites for gas exchange, such as the branchiostegites, may also augment lamellar gas exchange while buried (Chapter 4). In buried crabs, it is also possible that E_w% could tend to be overestimated during periods of reverse ventilation through the inclusion of hypoxic interstitial water in exhalent water samples. However, the extremely high values of E_w% recorded from reverse ventilating unburied crabs, could not be caused by such an effect, as inclusion of normoxic superincumbent water would tend to underestimate E_w%. Alternatively, these high values may indicate very high gill perfusion:irrigation ratios, as the highest values of E_w% were recorded at extremely low flow rates.

Thus, through behavioural and structural adaptations, buried *O. catharus* are able to obtain a sediment free and well-oxygenated inhalent respiratory water flow, regardless of which ventilatory mode is employed. Neither E_w% or $\dot{M}o_2$ were diminished in buried *O. catharus*, in either mode, when compared with unburied animals. In this species at least, reverse ventilation does not have to be utilised when buried in order to maintain respiratory function, as suggested by several previous studies. It would be of interest to investigate the effect of exostegal channel occlusion on ventilation in buried crabs.

Because of the variability in P_iO_2 recorded from buried forward ventilating *O. catharus*, some degree of caution is necessary when interpreting the values of $E_w\%$, $\dot{M}O_2$, and $\dot{V}_w/\dot{M}O_2$ shown by this group. Many species show a critical PO_2 (P_{crit}), at which point they change from being oxyregulators to oxyconformers, and $\dot{M}O_2$ declines with decreasing PO_2. P_{crit} values vary greatly between species. For example, *Cancer pagurus* shows a sudden decline in $\dot{M}O_2$ at a P_{crit} of between 60 and 80 torr (Bradford and Taylor, 1982), while the portunid, *Callinectes sapidus* appears not to have a P_{crit} at all, instead $\dot{M}O_2$ falls at a relatively constant rate at anything less than normoxia (Batterton and Cameron, 1978). In addition, considerable intraspecific variation has been noted, with temperature, activity, salinity, size, stage of the moult cycle, and time of the day all potentially altering P_{crit} (Herreid, 1980). Thus it is very difficult to assess whether or not fluctuating inspired oxygen tensions may have affected oxygen uptake in buried forward ventilating *O. catharus*. Although no relationship was found between $E_w\%$ and P_iO_2, $\dot{M}O_2$ declined significantly and $\dot{V}_w/\dot{M}O_2$ increased significantly with declining P_iO_2.

In Chapter 2, observations were made on the resting and postexercise ventilatory behaviour in buried and unburied *O. catharus*. When at rest, both buried and unburied individuals exclusively reverse ventilate. However, following 15 minutes of exercise by swimming, crabs began to utilise the forward mode (Chapter 2). Immediately following the exercise period, crabs remaining unburied spent 75% of the time, on average, forward ventilating, and 25% reverse ventilating. However, in crabs that were allowed to burrow, the proportion of time spent forward ventilating was much less at 33%. Both groups of crabs were exercised in an identical manner. Similar recorded increases in haemolymph [lactate] at the end of the exercise bout (Chapter 2) indicate the intensity of effort was similar and it is likely that the oxygen demands of the two groups at this time, would be comparable. Thus a similar ventilatory response would be expected in both buried and unburied crabs. Clearly this is not the case, suggesting burial had a modifying effect.

The results presented in table 4.1 show that, in unburied crabs, the convection requirement for reverse ventilation is higher than for forward ventilation. Therefore, to sustain a given $\dot{M}O_2$, the scaphognathites must pump a greater volume of water during reverse ventilation, increasing the energetic cost of ventilating in this mode. Thus, unburied animals may preferentially utilise the forward mode in order to lower the overall cost of ventilation during periods of high oxygen demand. As described above, a similar increase in $\dot{V}_w/\dot{M}O_2$ is seen in unburied *C. magister* when the direction of ventilation is reversed (McDonald et al., 1980). This results primarily from a fall in $E_w\%$ during reversals. As with *O. catharus*, the frequency of reversals is reduced during periods of high oxygen demand in unburied *C. magister*, thereby reducing the

energetic cost of ventilation (ie. the convection requirement). In buried *O. catharus*, the convection requirements of the two modes are similar and, from this perspective, no advantage would be gained from preferentially utilising either mode.

Ventilatory Work and Power

At any given flow rate, branchial chamber pressure was higher in buried animals than in unburied animals, with the values of P_{branch} being greatest in buried crabs utilising the forward ventilatory mode. The pressure generated is equal to the product of the flow and the resistance to flow. Thus the increased branchial chamber pressures recorded from buried animals suggest an increase in the resistance of the branchial water flow pathway. Consequently, the power and work requirements of ventilation are also increased when a crab is buried.

Resistance to ventilatory flow could be elevated in buried crabs via two main effects. Firstly, the ventilatory resistance may be increased by the passive effects of the sediment in which an animal is buried. Sediment settling around the branchial chamber apertures would be expected to increase the total resistance to water flow, resulting in greater values of P_{branch}. The resistance effects of the substrate may not be the same during forward and reverse ventilation, resulting in a valve-like action. When forward ventilating, sand grains would tend to be drawn to, and pack densely against setae fringing the inhalent branchial apertures at the bases of the legs, increasing the resistance greatly. In contrast, when the direction of flow is reversed, these apertures become exhalent and the sediment particles would tend to be pushed away from the openings allowing the exhalent stream to dissipate throughout the surrounding sediment without such a large increase in resistance. At high rates of reverse ventilatory flow, this effect is demonstrated as sand particles are held in suspension in the exhalent stream. This is seen as upwelling plumes of sand and water on the sediment surface (Chapter 2). As $\dot{V}_w$ falls, the plumes become smaller and smaller, until the sand grains settle out of suspension and the plumes disappear. Suspension of the sediment at high ventilation rates, may also directly reduce the resistance to flow presented by the substrate. However, when the pluming ceased an increase in P_{branch}, indicating a rise in resistance, could not be seen. These differential effects may partially account for the much higher pressures generated during periods of forward ventilation.

The second way that resistance to ventilatory water flow may be altered in buried crabs, is by the active regulation of the sizes and resistances of the various branchial apertures by the animal (Chapter 2). For example, upon burial, *O. catharus* raises the 3rd maxillipeds and holds them closely against the prostomial region of the carapace, so that, during reverse ventilation, the inhalent water stream is filtered by the dense setae on the margins of these appendages, and on the carapace. During forward ventilation, this would also pose a barrier to exhalent flow. When unburied, the 3rd maxillipeds are held in a lower position, separating the filtering setae and lowering the resistance to flow. Caine (1974) made identical observations on the use of the mouthparts in buried and unburied *Ovalipes guadulpensis*.

An ability to regulate the sizes of the other branchial openings, such as the M-E apertures, has been recorded for several species, including aquatic and terrestrial forms (eg. *Carcinus maenas* (Hughes et al., 1969) and *Ocypode saratan* (Eshky et al., 1990)). This appears to be achieved through movement of the mouthparts, specifically the 3rd maxillipeds. Caine (1974) suggested that *O. guadulpensis* could regulate the size of the M-E apertures via movements of the chelipeds. *O. guadulpensis* holds the chelipeds flexed against the pterygostomial region of the carapace when buried, as does *O. catharus*. According to Caine, this closes the M-E apertures, preventing sand from entering the branchial chambers (this was not directly observed). But in buried *O. catharus*, both exhalent and inhalent M-E water flow was demonstrated during forward and reverse ventilation, respectively (Chapter 2). In addition, the flexed position of the chelipeds assisted, rather than inhibited this flow, through the formation of the exostegal channels. The establishment of exostegal flow may enable buried crabs to actively lower the resistance of the water flow pathway by providing a clear channel between the sediment surface and the M-E apertures. In *Portumnus nasutus*, sand is excluded from these channels by anterolateral denticulations of the carapace, which act as a coarse sieve (Garstang, 1897b). *O. catharus* has 5 large anterolateral teeth on the carapace which, along with setae covering the pterygostomial regions of the carapace, presumably serve a similar function.

O. catharus can also alter the sizes and resistances of the three openings between the bases of the 4 pereiopods by dorso-ventral movements of the carapace (Chapter 2). The size of the large posterior branchial apertures between the 3rd and 4th walking legs can be varied greatly by this action. The posterior openings are major inhalent and exhalent sites during forward and reverse ventilation, respectively. Any change in the size of these openings would have significant consequences for gill irrigation and branchial resistance. In unburied reverse ventilating crabs, exhalent flow from the posterior openings was considerable, while flow from the M-E apertures was usually negligible. In contrast, buried reverse ventilating crabs showed substantial flow

from both the M-E openings and the posterior apertures. This may indicate a reduction in the size, and an increase in the resistance of the posterior openings, by depression of the carapace whilst buried, redirecting some exhalent water flow anteriorly to the M-E apertures. Fig. 3.9 shows a recording of $\dot{V}_w$, P_{branch} and F_r from a reverse ventilating buried animal which demonstrated brief alternating periods of exhalent flow from the M-E apertures and from the posterior apertures. Total flow remained constant, but P_{branch} increased greatly when the M-E apertures were the primary exhalent sites, indicating an increase in branchial chamber resistance, probably resulting from depression of the carapace and reduction in the size of the posterior branchial apertures. Changes in P_{branch} were observed over successive days in unburied crabs indicating active regulation of branchial chamber resistance was occuring in these animals also (Fig. 3.10). It was not possible to estimate the contributions of these active and passive mechanisms to total ventilatory resistance, but it is likely that both are involved.

Experimentally increasing the pressure head against which the scaphognathites had to generate flow (Series III), caused a reduction in scaphognathite stroke volume (V_s). The increased ventilatory resistance experienced by buried animals in the series II experiments had a similar effect. In unburied crabs, V_s was comparable in the two modes. However, buried crabs showed reduced values of V_s, particularly when utilising the forward mode and ventilatory resistance was greatest. Wilkens et al. (1984) connected specimens of *C. maenas* to a similar apparatus to that used in the present study for experimentally altering the total pressure difference (P_{total}) across the scaphognathites. These workers noted that when the P_{total} was increased to twice the P_{branch} (eg. P_{branch} = -2cm H_2O, P_{mask} = 2cm H_2O), $\dot{V}_w$ fell to zero. By comparison, it was found that in *O. catharus*, the scaphognathites were still able to generate flow in both the forward and reverse direction, at the same P_{total}. Predicted V_s at this pressure was 4.17 ml $beat^{-1}kg^{-1}$ in the forward mode and 4.74 ml $beat^{-1}kg^{-1}$ when reverse ventilating. This represents a reduction in V_s of only 22% and 25%, respectively, when compared with values of mean V_s from spontaneously ventilating crabs. Given the added resistance to ventilation in buried crabs, it is possible that this better ability to maintain ventilatory flow against a pressure head is the result of adaptation to a burrowing existence. It would be of interest to survey other brachyuran species in this respect.

An increase in $\dot{V}_w$, or the resistance to ventilation, necessitates an increase in the work and power output of the scaphognathite musculature when generating flow. Mercier and Wilkens (1984) showed that in *C. maenas*, stroke work done by the scaphognathites (W_s) increased proportionally to F_r raised to an exponent of approximately 1.5. As the stroke volume of the scaphognathites (V_s) is constant at all

values of F_r in this species, W_s would also be proportional to $\dot{V}_w$. In *O. catharus*, W_s was likewise, shown to increase with increasing $\dot{V}_w$, although the exponent of this relationship was not calculated. The scaphognathites also act as fixed stroke volume pumps in this species. Since the volume of water pumped with each beat is constant, the increase in W_s must result from an increase in the force generated by the ventilatory musculature, during each beat, at higher frequencies. This increased force would be required to overcome the greater branchial pressures seen at higher flow rates. Mercier and Wilkens (1984) showed that the peak isometric tension generated by the scaphognathites during the depression phase of the stroke, increased linearly with increasing F_r. Simultaneous EMG recordings from the depressor muscle D2A showed an increase in intraburst impulse frequency, presumably corresponding to increased motor neurone output to the muscle.

In performing the additional work, the oxygen requirements of the ventilatory muscles will be increased. From the literature, estimated values of the ventilatory fraction of total $\dot{M}o_2$ in decapod crustaceans vary greatly and all previous studies have used unburied forward ventilating animals. Assuming an efficiency of 10% for the scaphognathites, Batterton and Cameron (1978) estimated that as little as 0.02% of total $\dot{M}o_2$ was required for resting ventilation in *Callinectes sapidus*. Burnett and Bridges (1981) provided two quite different estimates of the ventilatory fraction of total $\dot{M}o_2$ in *C. pagurus* by using two methods of estimating $\dot{M}o_2$ during ventilatory pauses. The first value of 17% was obtained by comparing $\dot{M}o_2$ before and after a ventilatory pause. An increase in $\dot{M}o_2$ above prepause levels was observed following each period of apnoea, presumably indicating the repayment of an oxygen debt accrued during the pause. From the size of the debt and the duration of the pause, average $\dot{M}o_2$ during the pause, was estimated. The difference between this value and the prepause $\dot{M}o_2$ was assumed to be the oxygen cost of ventilation. However, as noted by Herreid (1980) and Ellington (1983), the observed pattern of $\dot{M}o_2$ recovery following a period of internal hypoxia is highly variable, and may not reflect the predicted oxygen defecit accurately, making this method somewhat unreliable. The second estimated value of 76% of total $\dot{M}o_2$ was obtained by estimating the $\dot{M}o_2$ from the fall in haemolymph oxygen content during a pause. This hinges on an accurate estimate of total haemolymph volume. In addition, a reduction was observed in cardiac frequency during apnoea, suggesting a fall in the oxygen requirements of this organ. Thus the energetic savings from reduced cardiac activity would be included in the cost of ventilation, overestimating the actual ventilatory fraction of $\dot{M}o_2$. These authors concede that the true oxygen cost of ventilation is likely to fall somewhere between their two estimates. Wilkens et al. (1984) estimated that ventilation in settled *C. maenas* required approximately 30% of the total $\dot{M}o_2$. This high cost was attributed

to a very low work efficiency of the scaphognathite pump (= hydrodynamic power output/metabolic energy input), which was estimated to be 3.15% at a $\dot{V}_w$ of 0.6 l $kg^{-1}min^{-1}$. This figure was obtained by eliminating the oxygen requirement of ventilation by dissecting out the scaphognathite and ventilatory musculature. Animals were then artificially ventilated and the difference in $\dot{M}o_2$ before and after scaphognathite ablation was considered to represent oxygen requirement of ventilation. This study is the most comprehensive attempt to assess the cost of ventilation in the brachyura. Using these efficiency values, the metabolic cost of ventilation in *O. catharus* was estimated. All calculations were made at a $\dot{V}_w$ of 0.6 l $kg^{-1}min^{-1}$. This requires a small extrapolation of the data for buried forward ventilating animals, as the highest recorded $\dot{V}_w$ from this group was 0.57 l $kg^{-1}min^{-1}$. One millilitre of O_2 was assumed to release 20.09 joules, the value given by Hill and Wyse (1989) for a mixed protein, carbohydrate and fat diet. The power output at the given $\dot{V}_w$ was calculated for each of the four treatment groups from regression analyses of log vs log plots of the data presented in Fig. 3.12.

For an unburied crab ventilating in the forward mode, the calculated W_r is 1.7 mW kg^{-1}, and at an efficiency of 3.15%, this corresponds to an oxygen requirement for ventilation of 7.2 μmol $kg^{-1}min^{-1}$. From Fig. 3.3, the estimated $\dot{M}o_2$ for an unburied 346g crab ventilating in the forward mode at 0.6 l $kg^{-1}min^{-1}$, is 59.68 μmol $kg^{-1}min^{-1}$. Thus ventilation consumes 12.1% of the total $\dot{M}o_2$. Ventilatory power requirements were similar in unburied reverse ventilating crabs (1.80 mW kg^{-1} = 7.8 μmol O_2 $kg^{-1}min^{-1}$), but because $\dot{M}o_2$ was lower in this group (33.12 μmol $kg^{-1}min^{-1}$), the proportion of total $\dot{M}o_2$ devoted to ventilation was considerably higher at 23.6%. When buried, the cost of ventilation was higher still. In buried reverse ventilating crabs both $\dot{M}o_2$ (78.22 μmol $kg^{-1}min^{-1}$) and W_r (3.69 mW kg^{-1} = 15.62 μmol O_2 kg^{-1} min^{-1}) were higher than in unburied animals utilising this mode, so that the proportion of $\dot{M}o_2$ devoted to ventilation was similar (20.0%). Upon switching to the forward mode the power output required at the given $\dot{V}_w$ was greatly increased to 10.8 mW kg^{-1} (45.7 μmol O_2 $kg^{-1}min^{-1}$), which would represent 43.3% of total $\dot{M}o_2$ (105.45 μmol $kg^{-1}min^{-1}$). It should be reiterated that these values are estimates only and that it is probable that the overall efficiency of the system is variable between treatment groups. For example, the smaller values of V_s found in buried crabs indicate a reduction in the mechanical efficiency of the scaphognathite pump. However, this may be offset by an increase in the efficiency of ventilation at higher power outputs as demonstrated in the teleost *Salmo gairdneri* (Jones and Schwarzfeld, 1974).

The higher calculated ventilatory fraction of total $\dot{M}o_2$ during reverse ventilation in unburied *O. catharus* does not result from a difference in the work and power requirements of ventilation in the two modes. Instead, this is a product of the reduced values of $\dot{M}o_2$ that occur when ventilation is reversed, and corroborates the previous suggestion that the energetic cost of this mode is increased as a result of a decrease in $E_w\%$ and increase in $\dot{V}_w/\dot{M}o_2$, as seen in table 3.1. However, in buried individuals of *O. catharus*, the cost of reverse ventilation, as a fraction of total $\dot{M}o_2$, is lower than of forward ventilation. This does not result from an improved $E_w\%$ or $\dot{V}_w/\dot{M}o_2$ when ventilation is reversed, but from an increase in work, power, and oxygen cost of ventilation in the forward mode. Therefore buried *O. catharus* may lower the overall energetic cost of ventilation by preferentially utilising the reverse mode.

Returning to the experiments of Chapter 2, as recovery from exercise progressed, the proportion of time spent utilising reverse ventilation increased in both buried and unburied animals, with shorter and less frequent bouts of forward ventilation occurring. When the animals were fully settled all crabs exclusively reverse ventilated regardless of burial state. This would be optimal in the buried group. However, as noted in Chapter 2, the state of being unburied and at rest is likely to be unnatural for *O. catharus*. Thus, exclusive reverse ventilation may be the only resting ventilatory strategy available and may be exhibited inappropriately in the latter.

Control of Ventilatory Switching

From table 3.1 it can be seen that changes in $\dot{M}o_2$ accompany ventilatory switching in both buried and unburied crabs. On average, $\dot{M}o_2$ was much lower during reverse ventilation, than during an adjacent period of forward ventilation. The fluctuations in $\dot{M}o_2$ in the unburied crabs, appear to result largely from differences in $E_w\%$ and $\dot{V}_w/\dot{M}o_2$ in the two modes. Whereas in buried animals, the observed fluctuations in $\dot{M}o_2$ appear to merely reflect differences in $\dot{V}_w$, since $E_w\%$ and $\dot{V}_w/\dot{M}o_2$ are similar in the two modes.

Measurements were obtained from two of the unburied crabs during complete forward-reverse-forward ventilatory cycles. At the onset of a reversal $\dot{V}_w$ and $\dot{M}o_2$ declined, then increased again to approximately pre-reversal levels with the recommencement of forward ventilation (pers. obs.). Thus $\dot{M}o_2$ was oscillating with each change in direction. These rapid fluctuations (periodicity = approximately 10 minutes) in $\dot{M}o_2$ presumably reflect changes in oxygen uptake at the gill, resulting from changes in $E_w\%$, rather than oxygen demand at the tissues. If the rate of oxygen

uptake falls during a reversal, but rate of utilisation by the tissues remains relatively constant, then the animal may incur an oxygen deficit. This could be offset by utilising the venous oxygen reserve during the reversal, resulting in a progressive drop in the venous Po_2 (P_vo_2) during a bout of reverse ventilation. Replenishment of the venous store of oxygen could be achieved rapidly upon returning to forward ventilation, as a result of an increased Po_2 gradient at the gill and a temporary improvement in E_w%. However, there was no evidence of this from the two forward-reverse-forward samples analysed (data not shown). It is possible the samples were not taken quickly enough following the return to forward ventilation to detect this.

Control of ventilatory switching may be achieved via such fluctuations in haemolymph oxygen tension. During reverse ventilation, Po_2 may fall to a threshold level, at which point forward ventilation is recommenced. The rapid brief bouts of reverse ventilation and predominance of the forward mode seen in unburied crabs immediately following exercise (Chapter 2) are consistent with this suggestion. Oxygen demand at this time would be high, thus P_vo_2 would be very rapidly depleted during a reversal. This would greatly limit the duration of each reversal. Burnett and Bridges (1981) proposed a similar mechanism for control of ventilatory pausing behaviour in *Cancer pagurus*. During a period of apnoea, the venous reserve of oxygen is utilised to meet the metabolic requirements. When internal Po_2 reaches a threshold level ventilation is recommenced and the venous reserve is replenished.

The observed pattern of ventilatory behaviour in unburied *O. catharus* following exercise, is very similar to that demonstrated in other brachyurans. Wilkens et al. (1974) identified a continuum of response of F_r to increasing stimulus frequency applied to command fibres in the circumesophageal connectives in *C. magister*. At low stimulus frequencies, high rates of forward ventilation were seen. As the stimulus frequency was increased, F_r began to fall and periods of reverse ventilation began to appear. At the highest frequencies, ventilatory pauses were induced. The same progression was recognised by McMahon and Wilkens (1977) in intact *Cancer productus* during recovery from apnoea. As with *O. catharus*, this progression appeared to be initiated by periods of high oxygen demand or low internal Po_2. These authors suggest that this pattern reflects an endogenous program that may be modulated by exogenous factors, such as haemolymph oxygen content.

The pattern of ventilatory behaviour in *O. catharus* allowed to bury following exercise, also conformed generally to the gradation of ventilatory response shown by unburied crabs (Chapter 2). The highest proportion of time spent forward ventilating was immediately following exercise, when F_r was highest. Utilisation of the reversed mode gradually increased, and finally pauses appearing, as F_r declined. This may again reflect the endogenous rhythm proposed by McMahon and Wilkens (1977).

However, the response of buried crabs was modified so that utilisation of the forward mode was greatly reduced when compared to unburied crabs (Chapter 2). A number of exogenous factors are likely to be responsible for this modification. Wilkens and McMahon (1972) showed that reversals in the lobster *Homarus americanus* could be reliably initiated by stimulation of the hairs bordering the lower margins of the branchiostegites. These authors suggest that particulate matter accumulating on these hairs during forward ventilation, could increase the resistance they present to ventilatory flow. As a result, the hairs may be progressively deflected by the water flow, eventually triggering a reversal to remove the detritus. Berlind (1977), using *C. maenas* suggested that 5-hydroxytryptamine (5HT) was the neurotransmitter in this reflex pathway. Increasing doses of 5HT stimulated an increase in the frequency and duration and frequency of reversals, and at the highest doses prolonged reversals were elicited. In addition, the increased frequency of reversals stimulated by particulate matter in the inhalent stream could be blocked using antagonists of 5HT action. The branchial chamber apertures in *O. catharus* are surrounded by dense setae. Thus, it is possible that in buried crabs, these hairs are rapidly deflected during forward ventilation as a result of the surrounding substrate particles being drawn toward the branchial chamber apertures. This may provide strong a stimulus to reverse the direction of ventilation and contribute to the reduction seen in the utilisation of the forward mode.

Other factors, such as changes in P_{branch}, or internal and external Po_2 may help mediate the response in buried crabs. For example, the large increase in P_{branch} seen during forward ventilation may also serve to stimulate reverse ventilation. When ventilation is reversed, P_{branch} would drop and the stimulus would be removed.

Taylor and Butler (1973) showed that prolonged periods of reverse ventilation may be related to changes in internal Po_2 in the portunid *Carcinus maenas*. In normoxic conditions, aquatic ventilation is typically in the forward mode. However, as internal and external Po_2 decline, the animals raise the mouthparts above the water surface and ventilate the gills in the reverse direction with air, thus maintaing a high P_io_2. The emersion response of *C. maenas* is remarkably similar to the burial response of *O. catharus*. Depression of internal Po_2 could potentially occur in buried *O. catharus*. When utilising the forward mode, V_s is greatly reduced. This may limit the ability to generate ventilatory flow, compromising oxygen uptake at the gill. In addition, the increased work and power requirements of forward ventilation when buried, increase the oxygen demands of the ventilatory musculature. This may also serve to lower the venous reserve. Wilkens et al. (1984) showed that changes in the ventilatory oxygen requirement in *C. maenas*, could influence P_vo_2, when ablation of the scaphognathite musculature resulted in an increase in the venous oxygen tensions.

The predominate mode of ventilation in *O. catharus* differs according to the animals physiological state and environmental cues. This species has specific adaptations that permit it to alter utilisation of the two ventilatory modes, presumably depending on extrinsic and intrinsic factors, in order to reduce the energetic cost of ventilation. Burial and exercise appear to stimulate conflicting responses by increasing the utilisation of reverse and forward ventilation, respectively. It is inferred that, when buried at rest, reverse ventilation is utilised by *O. catharus* as an adaptation to reduction of the fraction of total $\dot{M}o_2$ that must be devoted to generating ventilatory flow. Interestingly, reverse ventilation is the predominate mode in settled unburied crabs, when forward ventilation would be less costly. This may simply indicate that reverse ventilation has become the "normal" mode, associated with prolonged periods of burial in sandy substrates. In its natural habitat, it is unlikely that *O. catharus* would be found at rest on the surface of the substrate. Thus the observed ventilatory response to this situation under experimental conditions may reflect an inappropriate response to a circumstances not normally encountered in the wild. Overall it appears that the relative energetic costs of the ventilatory modes under different conditions primarily determines the preferred mode, rather than activity or burial state as previously suggested.

Chapter 4

The Circulatory System of the New Zealand Paddle Crab Ovalipes catharus with particular reference to the Vasculature of the Branchiostegites.

Abstract

The venous circulation of the branchiostegites is extensive and well developed in *O. catharus*. Haemolymph enters this vascular bed from the anterior hepatic sinuses and returns directly to the pericardium without passing through the gills. The arrangement of vessels is very similar to that described for other species, including amphibious forms. The branchiostegal sinus is a thin sheet of small (200-300 μm) interconnecting sinuses and lacunae. There appear to be two routes of haemolymph return to the pericardium from this region. Large collecting vessels are found bordering the posterior margin of the carapace and these connect to the pericardium posteriorly, while other collecting vessels drain into the pericardium laterally. The general circulation is described and a possible role for the branchiostegites as alternative sites to the gills for respiratory gas exchange in *O. catharus*, is discussed.

Introduction

In most aquatic decapods the gills are the primary sites of respiratory gas exchange. The gills arise from the body wall on or near the point of attachment of the thoracic appendages. A number of specific adaptations are seen which increase the efficiency of gas exchange via these organs. Water currents are drawn through the branchial chambers and past the gills by the rhythmic beating of the scaphognathites. Various arrangements of plate-like or finger-like extensions of the body surface increase the available surface area of the gills for gas exchange. While most of the animal is covered in a thick and virtually impermeable cuticle, the cuticular thickness of the gills is greatly reduced, decreasing the distance for diffusion of respiratory gases into and out of the haemolymph. Richards (1992) calculated the harmonic mean thickness of the lamellar cuticle and epithelium in *O. catharus* to be 2.11 μm.

In semi-terrestrial and terrestrial decapod species, the role of the gills in respiration may be reduced or eliminated altogether. Instead the branchiostegal lining of the branchial chambers has become highly vascularised and, in some terrestrial species, the surface area of this region may be increased through the development of folds, corrugations or tufts to form lungs (McMahon and Burggren, 1988). The origins of the complex vascular beds of the lungs of terrestrial crabs can be traced back through semiterrestrial species, such as *Hemigrapsus nudus* (Greenaway and Farrelly, 1990), to fully aquatic brachyurans, like *Cancer productus* (McMahon and Burnett, 1990). But a thorough investigation of the branchiostegal vasculature in an aquatic crab has yet to be made.

Richards (1992) suggested that *O. catharus* may utilise sites, other than the gills, for respiratory gas exchange. This was based upon the observation that the calculated capacity of the gills for the diffusion of oxygen into the haemolymph was much less than the measured oxygen diffusion into whole animals. In addition, despite apparently poor irrigation of the gills during reverse ventilation, oxygen extraction remains high (chapter 2). The most likely alternative site for gas exchange appears to be the branchiostegite.

Vascular corrosion casting techniques were employed to examine the general circulation of *O. catharus*. The extent of the vascularisation of the branchiostegite was investigated specifically to evaluate the suggestion that this region may function as an alternative site for respiratory gas exchange.

Methods and Materials

Medium sized crabs of both sexes were used. Injection holes were drilled through the carapace of each animal using a dentists drill. Depending on which areas were to be filled, holes were either drilled in the carapace overlying the pericardium, eye sinuses or hepatic sinuses (see below). Pressure relief holes were also made in the arthrodial membranes and cuticle on the efferent side of the structures to be filled. This helped prevent excessive haemocoelic pressures and subsequent damage of vascular structures during injection of the polymer. It also helped direct the polymer to areas of interest. In some animals unwanted portions of the circulation were filled by injection of molten agar or petroleum jelly prior to injection of the plastic.

A quantity of Batson's No. 17 Anatomical corrosion compound (Polysciences Inc.) was prepared as directed. The viscosity of the polymer was reduced by the addition of methyl methacrylate monomer at a ratio of monomer to polymer of 1:4. This mixture was placed in a 20 ml plastic syringe. A short length of large diameter cannula with a 16 G needle at either end was connected to the syringe. The cannula was inserted into a live crab and the polymer was injected by hand. Injection of methacrylate into the pericardium invariably filled most of the arterial system, but also some of the major sinuses, especially the eye sinuses, hepatic sinuses and some of the branchiostegal sinus. The efferent side of the branchial circulation was often back-filled by this method. Injection into the large eye sinuses produced satisfactory venous casts of the hepatic sinuses, ventral sinuses of the head region and much of the branchiostegal region. To fill the branchiostegal circulation plastic was injected into the sinuses of the hepatopancreas. The injection pressure was not monitored or controlled.

Following injection, the holes were sealed with silicone grease and the crabs were immersed in water overnight to allow for the dissipation of heat as the plastic cured. The carcasses were then placed in a 12.5 M NaOH solution with EDTA at 50°C to dissolve the soft tissues and exoskeleton. The carcasses were periodically rinsed with tap water to assist the removal of the tissues. Soft tissues dissolved completely but only partial dissolution of the exoskeleton could be achieved, the remainder was carefully dissected away from around the cast with fine forceps.

Casts were photographed whole and then dissected to show particular structures more clearly, these areas were also photographed. Portions of the casts were dissected out, mounted on aluminium stubs with conductive carbon paint and sputter coated with gold to a thickness of approximately 40 nm. These were then examined with a Cambridge Stereoscan 250 mkII scanning electron microscope at an accelerating voltage of 20 kV.

Results and Discussion

General circulation

The decapod heart is a large single chambered muscular organ often referred to as the ventricle. It is held suspended within the dorsal pericardium by a number of suspensory alary ligaments. In *O. catharus* the position and size of the pericardium is outlined by a series of marks on the dorsal carapace (Fig. 4.1). The entire structure appears roughly star-like in shape when viewed dorsally. The anterior margin of the pericardium is indicated by the dark "butterfly" mark, and the lateral boundaries by two semilunar depressions in the carapace. Posteriorly, the pericardium extends toward the carapacial margin. Many of these marks probably correspond to muscle attachment points. For example, the semilunar depressions overly the insertions of the epimeral attractor muscles onto the carapace. The pericardium is thought to act as a second chamber of the heart by priming the ventricle (McMahon and Burnett, 1990).

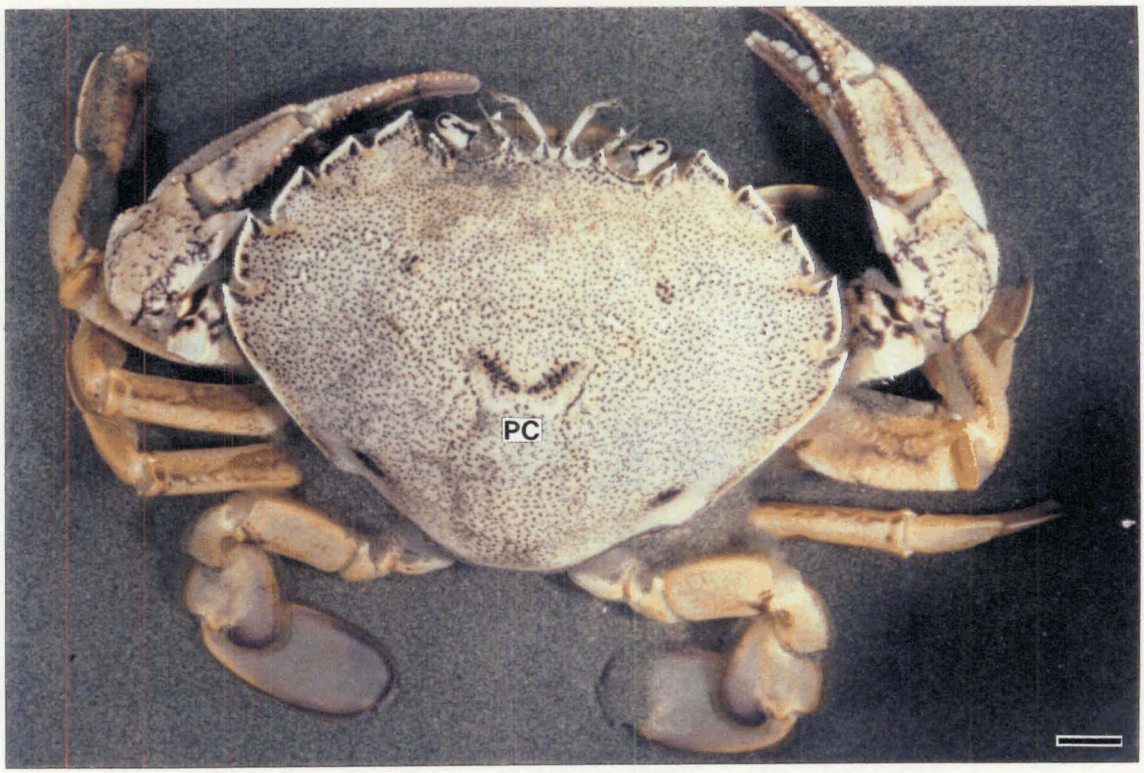

Fig. 4.1. Dorsal view of *O. catharus* showing the markings on the carapace outlining the position of the pericardium (PC). Scale bar = 1 cm.

At diastole the ventricle fills with haemolymph collected in the pericardial cavity, via three pairs of valved ostia. One pair is situated dorsally, one pair laterally on each side of the heart and the third pair are on the postero-lateral surface of the heart (Fig. 4.2a). Each ostium has two semilunar valves which prevent the reflux of haemolymph into the pericardium at systole, thus maintaining unidirectional flow (Fig. 4.2b). The ventricular walls are comprised of an intricate arrangement of muscular trabeculae to enable efficient cardioejection (Maynard, 1960) (Fig. 4.2c).

Haemolymph is ejected from the heart into seven arteries supplying the systemic circulation. These arteries are also valved, at their origins, preventing reflux into the heart (McMahon and Burnett, 1990). Five of the seven arteries leave the heart anteriorly. The anterior aorta extends anteriorly along the dorsal midline of the animal. This vessel supplies haemolymph to the cerebral ganglion (brain) and eyes (Fig. 4.3a). A pair of arteries arise from the ventricular wall on either side of the anterior aorta (Fig. 4.3b). These are the lateral anterior arteries (also referred to as the antennal arteries by McMahon and Burnett, 1990). These supply the foregut, branchiostegite, pterygostomial region, antennal glands and cephalic appendages (antennae and antennules). The large paired hepatic arteries originate from the ventricle ventrally to the anterior lateral arteries. These vessels perfuse the large lobes of the hepatopancreas, also the foregut and midgut (Fig. 4.3a).

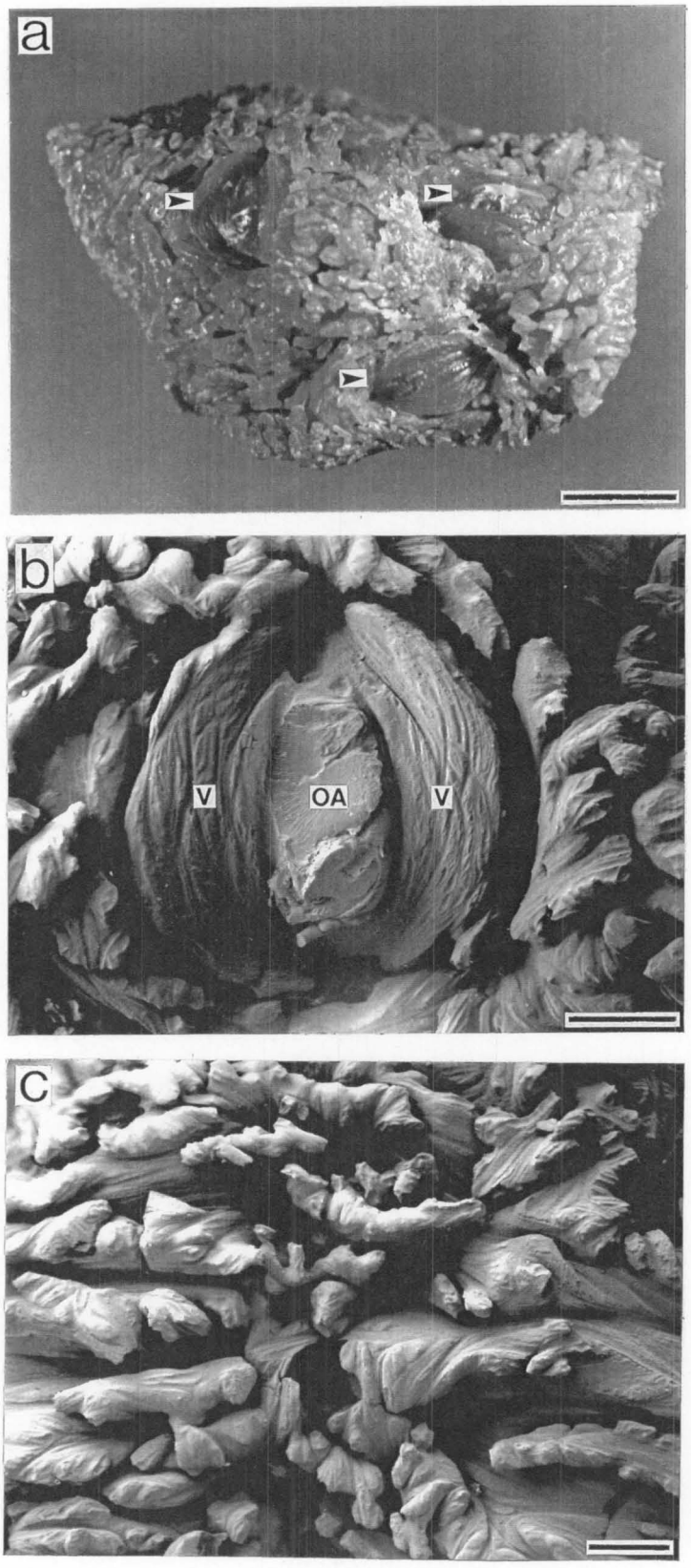

Fig. 4.2. a) Left lateral view of a cast of the ventricle of *O. catharus*. The arrows indicate the positions of the ostia. The anterior end is to the left of the picture. Scale bar = 0.5 cm. b) A scanning electron micrograph of a cast of one ostium. The aperture of the ostium (OA) is seen as a solid plug of plastic and the impression of the two semilunar valves (V) can be seen. Scale bar = 1 mm. c) A scanning electron micrograph of a cast of the dorsal wall of the ventricle showing the complex arrangement of muscular trabeculae within the wall. Scale bar = 1 mm.

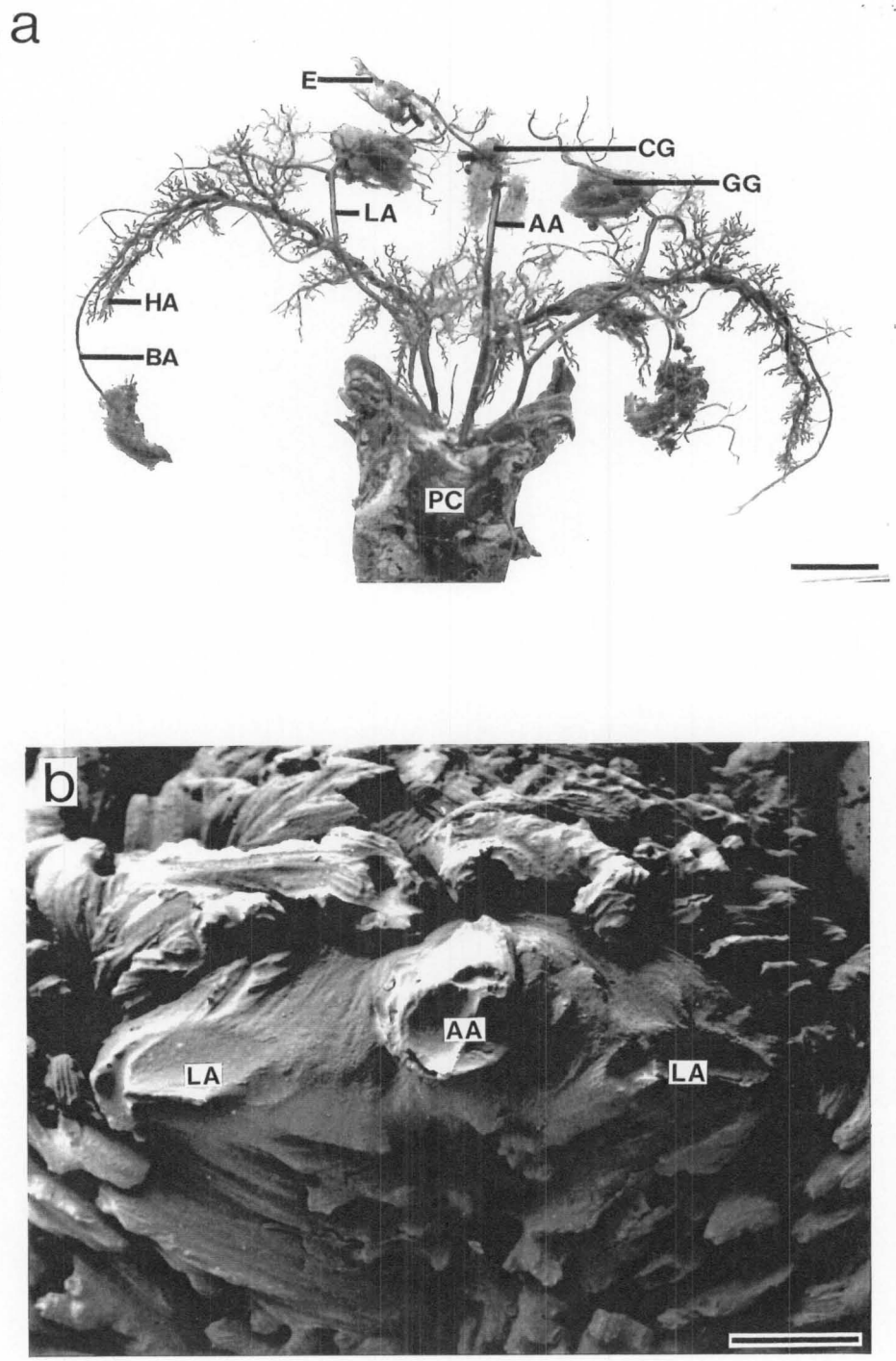

Fig. 4.3. a) Dorsal view of a cast of the anterior arterial circulation. PC = pericardium, AA = anterior aorta, HA = hepatic artery, LA = lateral antennal artery, BA = branchiostegal artery, GG = green gland, CG = cerebral ganglion, E = eye. Scale bar = 1 cm. b) Scanning electron micrograph of a cast of anterior wall of the ventricle showing the origin of the anterior aorta (AA) and paired lateral antennal arteries (LA). Scale bar = 1 mm.

The largest artery in *O. catharus*, is the sternal artery. This vessel descends from the ventral surface of the ventricle. If a crab is viewed ventrally, the sternal artery descends to a point underlying the tip of the last abdominal segment where it branches radially and laterally into eight smaller vessels (Fig. 4.4a). McLaughlin (1983) stated that, in the brachyura, the sternal artery divides into two vessels only; the ventral thoracic artery, supplying the mouthparts, including the scaphognathites, and the posterior ventral abdominal artery. In *O. catharus*, at the point where the ventral thoracic artery originates, three pairs of vessels branch radially (Fig. 4.4a). Numbering from anterior to posterior, the first and second vessels on each side supply the chelipeds and first pereiopods, respectively. The third vessel on either side proceeds postero-laterally for a short distance before splitting into three, delivering haemolymph to the 2nd, 3rd and 4th pereiopods. The eighth vessel originating from this junction is much smaller than the others and proceeds postero-dorsally, lying closely behind the sternal artery for much of its length, before diverging posteriorly and supplying the abdomen. This is the posterior ventral abdominal artery (Fig. 4.4b).

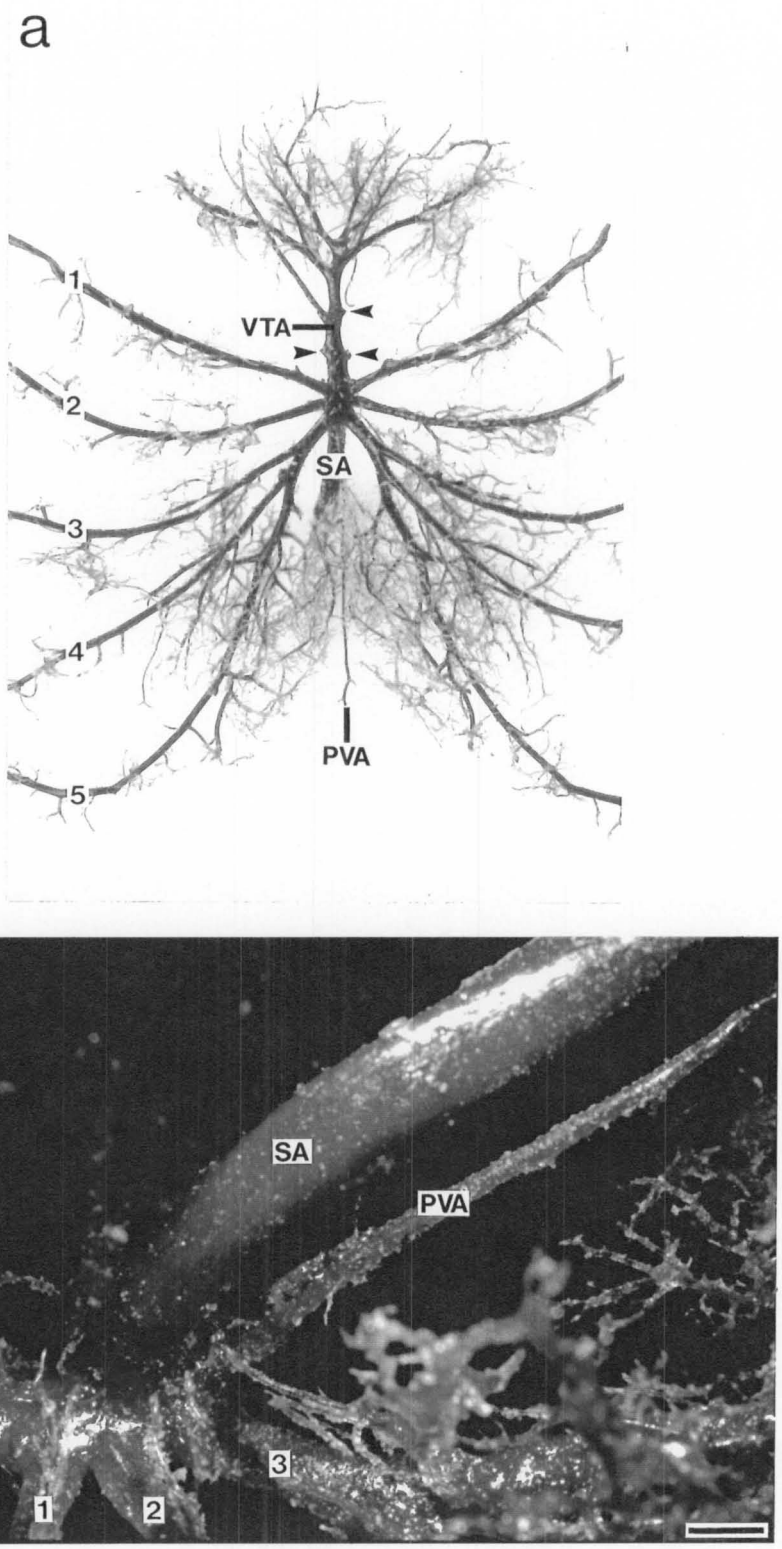

Fig. 4.4. a) A ventral view of a cast of the ventral arterial circulation in *O. catharus*. SA = sternal artery, VTA = ventral thoracic artery. The arteries numbered 1 - 5 indicate the vessels supplying the chelipeds (1) and four pairs of perieopods (2 - 5). Note that three arteries are missing from the cast, indicated by the three arrows. The two posterior arrows indicate the positions of the arteries supplying the third maxillipeds while the single arrow to the right of the VTA indicates a missing artery to the second maxilliped. Scale bar = 1 cm. b) A closeup view of the junction at which the sternal artery (SA) divides showing the relative positions of the posterior ventral abdominal artery (PVA) and arteries supplying the chelipeds (1) and four pereiopods (2 and 3). Scale bar = 1 mm.

The single vessel leaving the ventricle of heart posteriorly, is the posterior aorta or superior abdominal artery. As this vessel passes down the midline of the abdomen, paired segmental arteries arise on each side. These secondary vessels are probably longer in females which have a much broader abdomen, but this was not demonstrated in casts due to incomplete perfusion of the abdomen. This would imply secondary sexual development of the vascular system at the onset of maturity. The posterior aorta was very difficult to perfuse with methacrylate and was filled on only one cast. This may partially reflect the low metabolic requirements for oxygen, and thus haemolymph, of the abdomen, which is greatly reduced in the brachyura. This also supports the observation of Pike (1947, in McLaughlin, 1983) that the ventral abdominal artery, rather than the posterior aorta, has become the major vessel supplying the abdomen in the brachyura.

The major arteries continue to branch into progressively smaller vessels in the body tissues. Maynard (1960) designated vessels lined with a single layer of endothelium, that were between 7 and 50 μm in diameter, as capillaries. As noted by McMahon and Burnett (1990) the density of capillaries varies greatly in different tissues and organs, with neural tissue, antennal glands, hepatopancreas, and digestive organs having the highest densities. Fig. 4.5a and b show the dense network of capillaries and haemolymph spaces within the cerebral ganglion, or brain. The terminal end of anterior aorta is distended to form a chamber which acts as an auxiliary heart, this is the cor frontale (Steinacker, 1978). Within the lumen of the chamber lie two strips of longitudinal striated muscle which contract to facilitate the delivery of haemolymph to the brain, via the cerebral artery, and to the eyes, via the paired ophthalmic arteries. Fig. 4.6a shows the terminus of the arterial supply to the antennal gland. Each gland is composed of two main parts: the apical coelomosac, or end sac, and the labyrinth. In *Callinectes sapidus* the two portions are closely associated forming a spongy green mass. The end sac is situated dorsally and centrally, with the labyrinth occupying the periphery and ventral parts (Johnson, 1980). This also appears to be the case in *O. catharus*. The end sac is directly supplied with arterial haemolymph by the antennal arteries (Fig. 4.6a) and fluid collects in the lumen by filtration from the haemolymph. The wall of the end sac interdigitates with the wall of the labyrinth. The two are closely applied but between them there are haemal spaces fed by the antennal artery (Johnson, 1980). The wall of the labyrinth is glandular and complexly folded and is thought to be responsible for the reabsorption of proteins and for moving water isotonically across the epithelium. The highly irregular wall of the lumen presumably maximises the surface area available for such a purpose (Fig. 4.6b). The lumen of the labyrinth connects to a large bladder via an elongated nephridial canal. The bladder opens to the environment via a duct exiting near the base of each antenna, which is covered by a movable operculum.

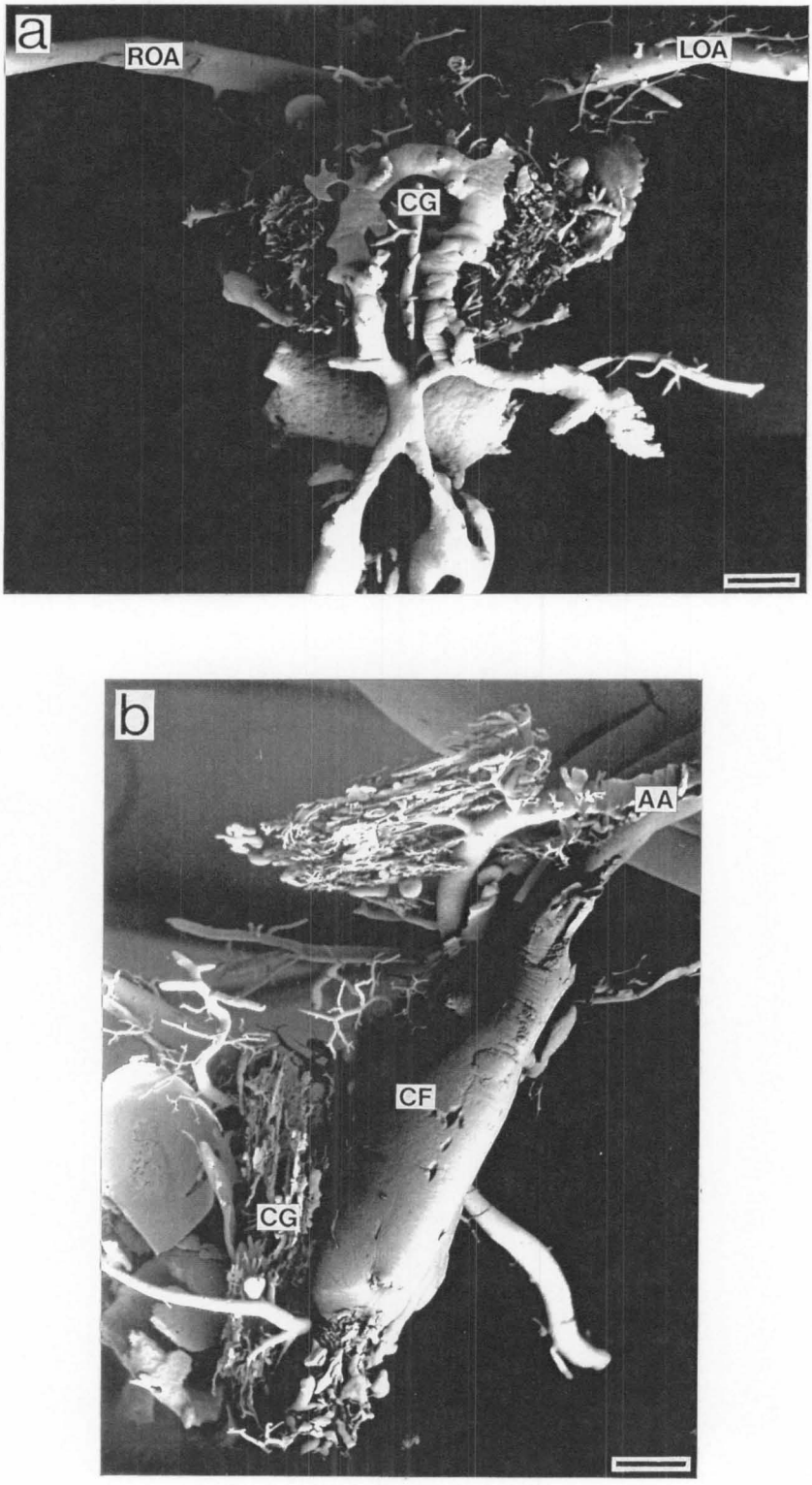

Fig. 4.5. Scanning electron micrographs of vascular casts of the cerebral ganglion (brain) of *O. catharus*. a) An anterior view of the cerebral ganglion (CG) showing bilateral symmetry. b) A left lateral view. The distended terminus of the anterior aorta (CF) is the auxiliary heart, the cor frontale. AA = anterior aorta, LOA = left ophthalmic artery, ROA = right ophthalmic artery. Scale bars = 1 mm.

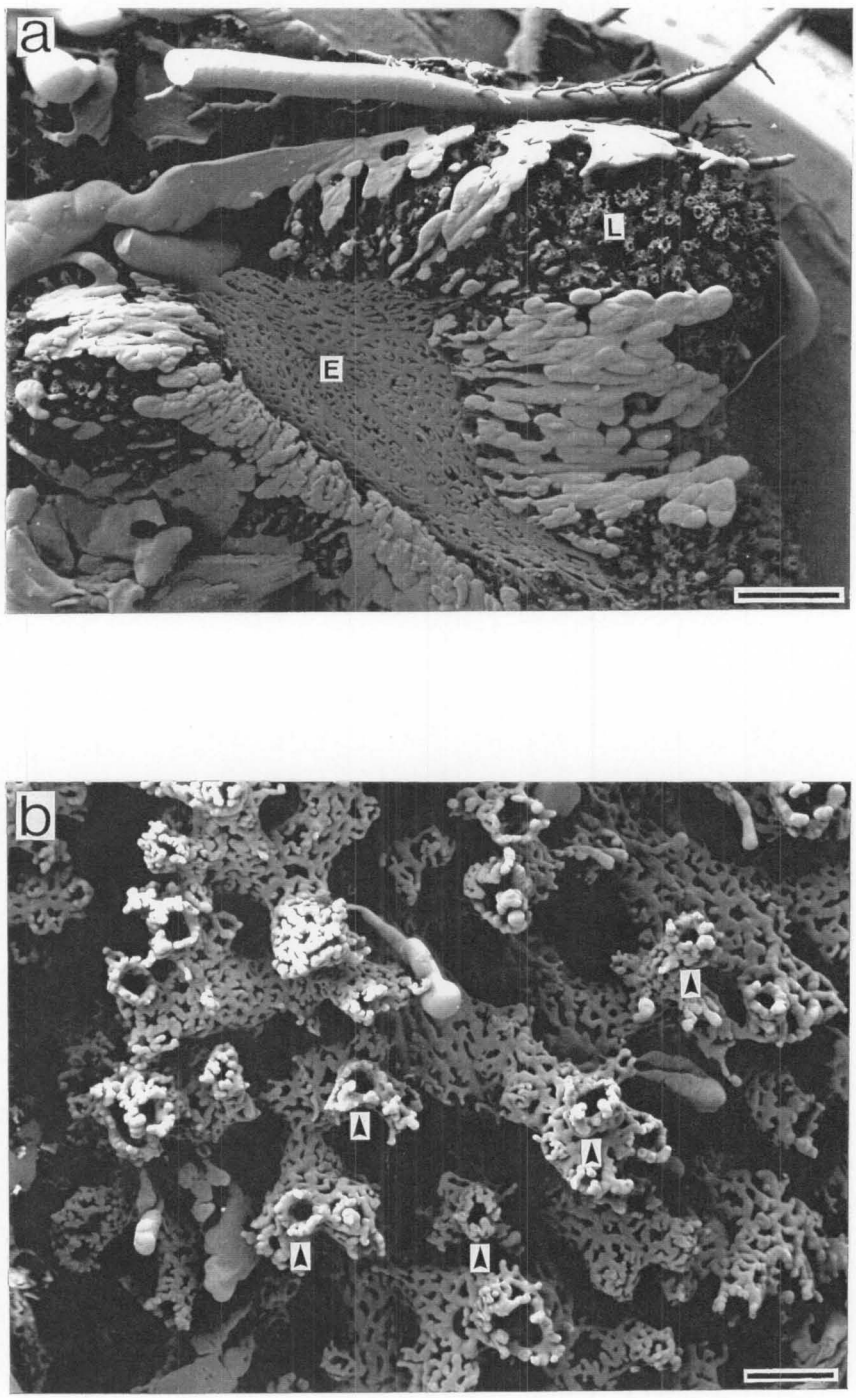

Fig. 4.6. Scanning electron micrographs of vascular casts of the antennal gland. a) The arterial vessels supplying the end sac (E) are seen on the dorsal surface of the cast. The antennal artery continues into the organ to feed the haemal spaces lying between the interdigitating walls of the endsac and the labyrinth (L). Scale bar = 1 mm. b) Close up of the haemolymph vessels in the wall of the labyrinth showing the highly folded nature of this tissue. Duct-like invaginations of the lumen are arrowed. Scale bar = 200μm.

The fine capillaries drain into small irregular lacunae and sinuses that are not bounded by any type of membrane, so that the haemolymph is in direct contact with the tissues. It is the lack of a distinct venous circulation that makes the crustacean circulatory system an open system. There are sinuses associated with all the major vessels, to allow draining of the haemolymph from the arterial system. The sizes and shapes of these spaces are primarily determined by the shape of the surrounding organs or tissues. For example, in the limbs the sinuses are long tubular structures generally running longitudinally within the limb. Movement of haemolymph within these spaces may be facilitated by the action of the locomotory muscles. In *O. catharus* there are also ventral sinuses arranged radially in the pleural muscle compartments, similar to the arrangement of the radially branching arteries arising from the sternal artery. These haemolymph spaces are the pleural sinuses described by Greenaway and Farrelly (1984) in *Ocypode cordimanus*.

Description of the venous side of the circulation is very difficult due to the irregular shape and complicated interconnection of the sinuses, so I will describe the major sinuses only. Immediately posterior to the eyes and surrounding the cardiac stomach are two large sinuses (Fig. 4.7a), these are referred to as the eye sinuses in *O. cordimanus* by Greenaway and Farrelly (1984). These surround the stomach entirely and are connected above and below this organ. The eye sinuses communicate laterally with the hepatic sinuses that collect haemolymph from the capillary beds in the lobes of the hepatopancreas. When viewed dorsally, the hepatic sinuses are large and crescent shaped, mimicking the shape of the hepatopancreatic lobes (Fig 4.7b). Ventrally, the eye sinuses connect with a complex arrangement of small interconnecting sinuses surrounding the mouthparts and ventral nervous system. The sinuses in the head region extend posteriorly below the pericardium and connect with the radiating ventral pleural sinuses that drain haemolymph from the musculature of the pereiopods.

Fig. 4.8a gives a lateral view of the hepatic sinus with the space occupied by the hepatopancreas visible. Along the posterior wall of this space, a number of vertical compartments can be seen. Within each of these compartments lie the anterior dorsoventral muscles (DVM) (Taylor et al., 1992). These muscles insert on the dorsal carapace and the flexible roof of each branchial chamber and are involved in the regulation of haemocoelic pressure (see below). Anteriorly the hepatic sinuses are dorsoventrally thickened, as they envelop the hepatopancreas, but posteriorly the thickness of the sinus is reduced as it becomes the thin branchiostegal sinus and passes over the branchial chamber (Fig. 4.8b & c).

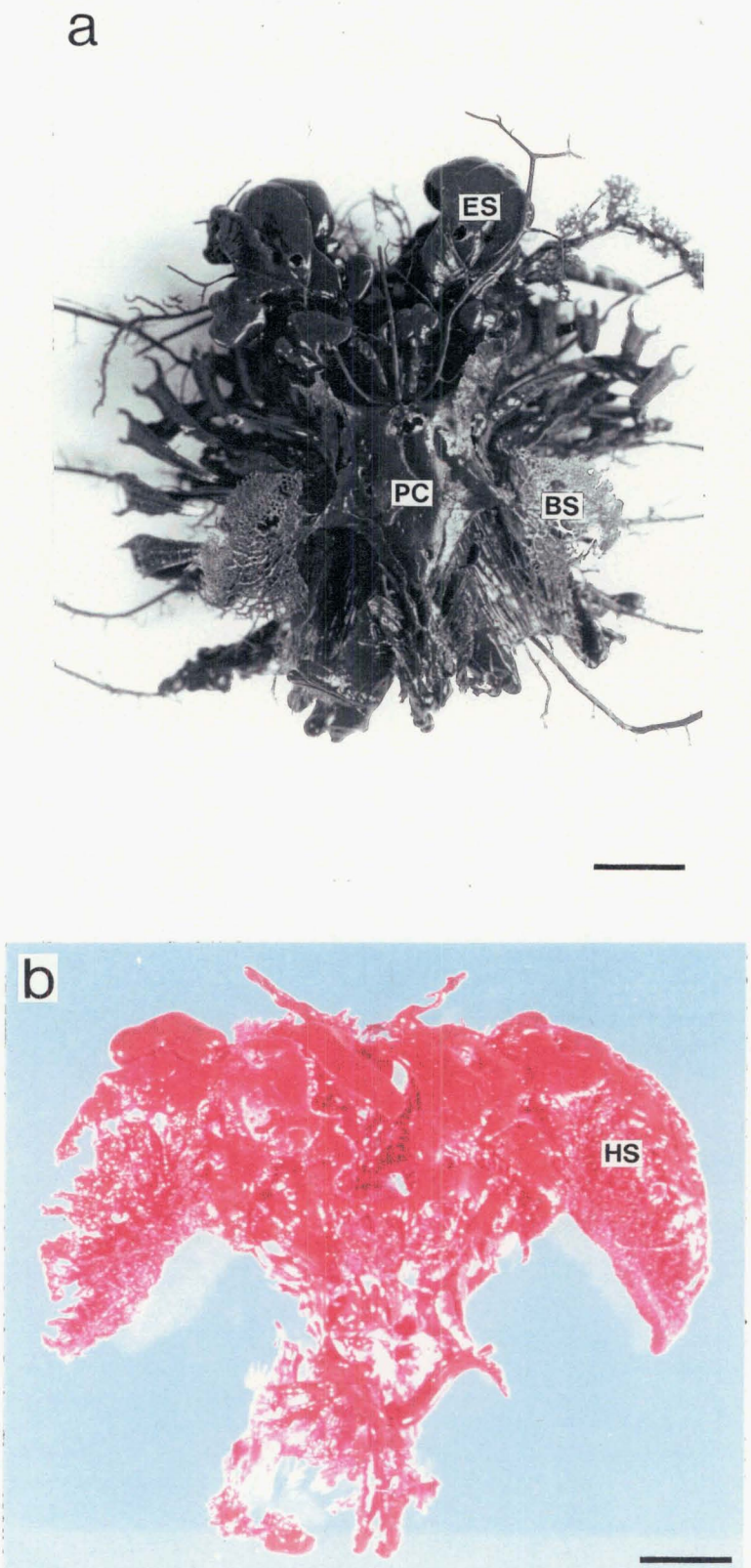

Fig. 4.7. a) Dorsal view of a whole animal cast of the general circulation in *O. catharus*. ES = eye sinus, PC = pericardium, BS = branchiostegal sinus. b) Dorsal view of a venous cast showing the large hepatic sinuses (HS). Scale bars = 1 cm.

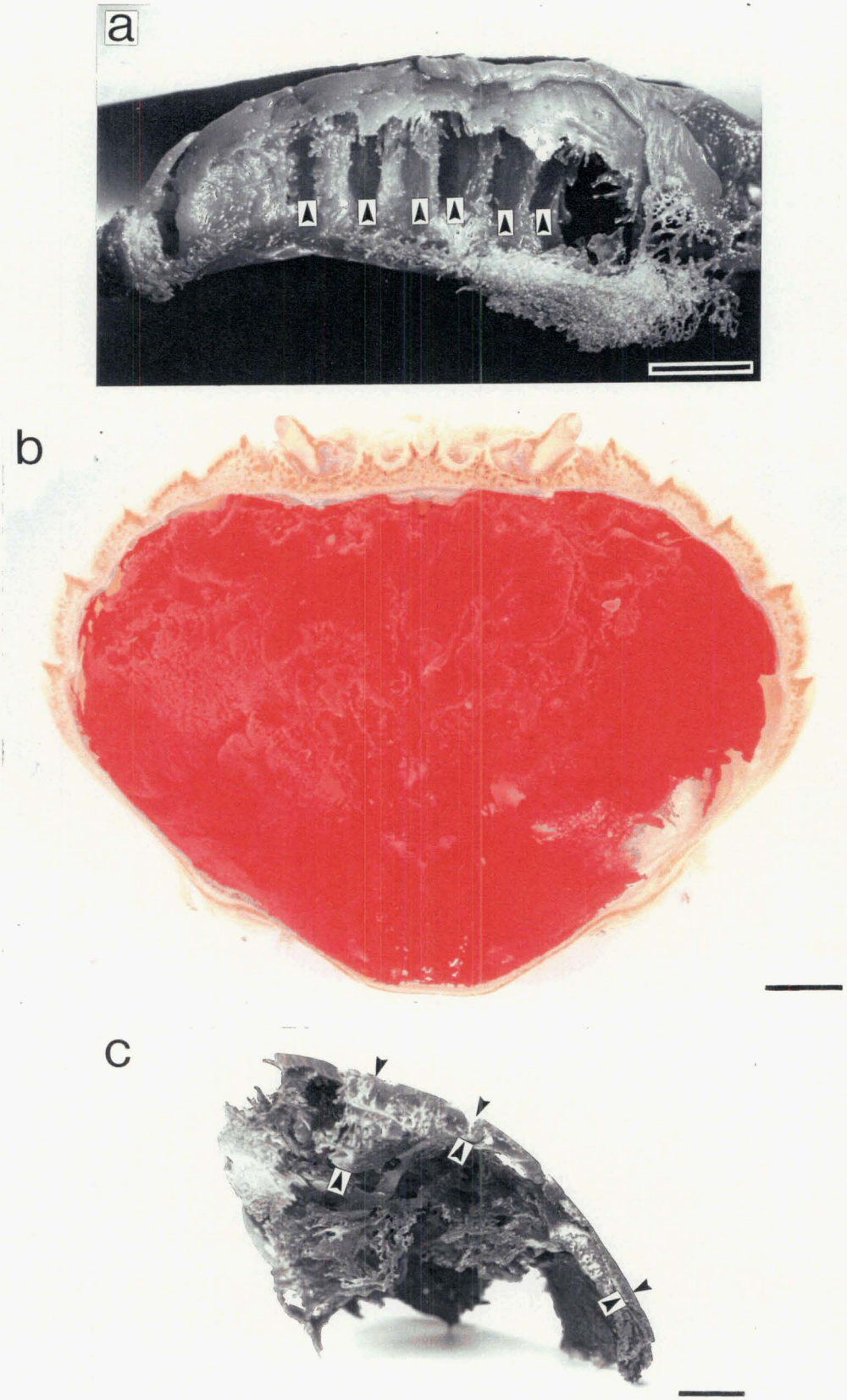

Fig. 4.8. a) A lateral view of the space in a cast of the hepatic sinus occupied by the lobe of the hepatopancreas. Scale bar = 0.5 cm. The arrows indicate the six vertical compartments visible where the anterior dorsoventral muscles are located. b) A dorsal view of a whole animals cast showing the extent of the branchiostegal sinus overlying the branchial chambers. Scale bar = 1 cm. c) A lateral view of the same cast in (b) after being sectioned longitudinally, demonstrating the varying thickness of this haemal space. Arrows indicate thickness. Scale bar = 1 cm.

As the branchiostegal sinus becomes thinner posteriorly, the organisation of the blood spaces changes to become a sheet of progressively smaller interconnecting sinuses with diameters of about 200 - 300 μm (Fig. 4.9a). This arrangement of small sinuses lines the posterior half of the dorsal roof of each branchial chamber, and also most of the ventral carapace (pterygostomial region etc.). The small vessels, as applied to the dorsal branchiostegite, appear to be roughly arranged in interconnecting rings, with lacunae projecting into the spaces between the rings. Larger tubular collecting sinuses (diameter approximately 500 μm) returning haemolymph to the pericardium were found bordering the posterior margin of the carapace (Fig. 4.9b). There is also an increase in the size and order of the branchiostegal vessels lateral to the pericardium (Fig. 4.10). These also appear to be collecting vessels which drain into the pericardium.

The flattened appearance of the vessels in the branchiostegites in many of the micrographs (eg. Fig. 4.10) may be an artifact of the casting procedure. The roof of the branchial chambers appear to have dropped onto the dorsal surfaces of the gills following injection of the plastic, probably because the chambers were filled with air rather than water.

The appearance of casts of the branchiostegal circulation in *O. catharus* is very similar to that of casts of the "lung" of the amphibious crab *Hemigrapsus nudus* (Greenaway and Farrelly, 1990). A similar arrangement of posterior marginal collecting vessels is apparent in the latter, which these authors refer to as the "pulmonary veins". Three routes of haemolymph return from the branchiostegite to the pericardium are identified in *Hemigrapsus nudus*, as opposed to two in *O. catharus* (posterior and lateral). In the present study, a single cast of the circulation of the subtidal rock crab *Cancer novazealandiae* was made which also showed well developed tubular sinuses bordering the ventral and posterior margins of the carapace and these connected to the pericardium laterally. In this cast, the distal ends of fine vessels lining the branchiostegites were also back-filled from the marginal collecting vessels, suggesting a similar branchiostegal circulation to that found in *O. catharus*.

Haemolymph entering the branchiostegal sinus does not pass through the gills before reaching the pericardium. Typically, the gills are the primary sites of oxygenation of the haemolymph in aquatic species. Thus if no gas exchange occurs across the branchiostegal surfaces (see below), the haemolymph returning to the heart from the branchiostegites will be relatively hypoxic and will lower the arterial Po_2 to the systemic circulation. However, in addition to the being supplied with venous haemolymph from the hepatic sinuses, the branchiostegal sinuses also receive oxygenated haemolymph via the branchiostegal arteries (Fig. 4.3a).

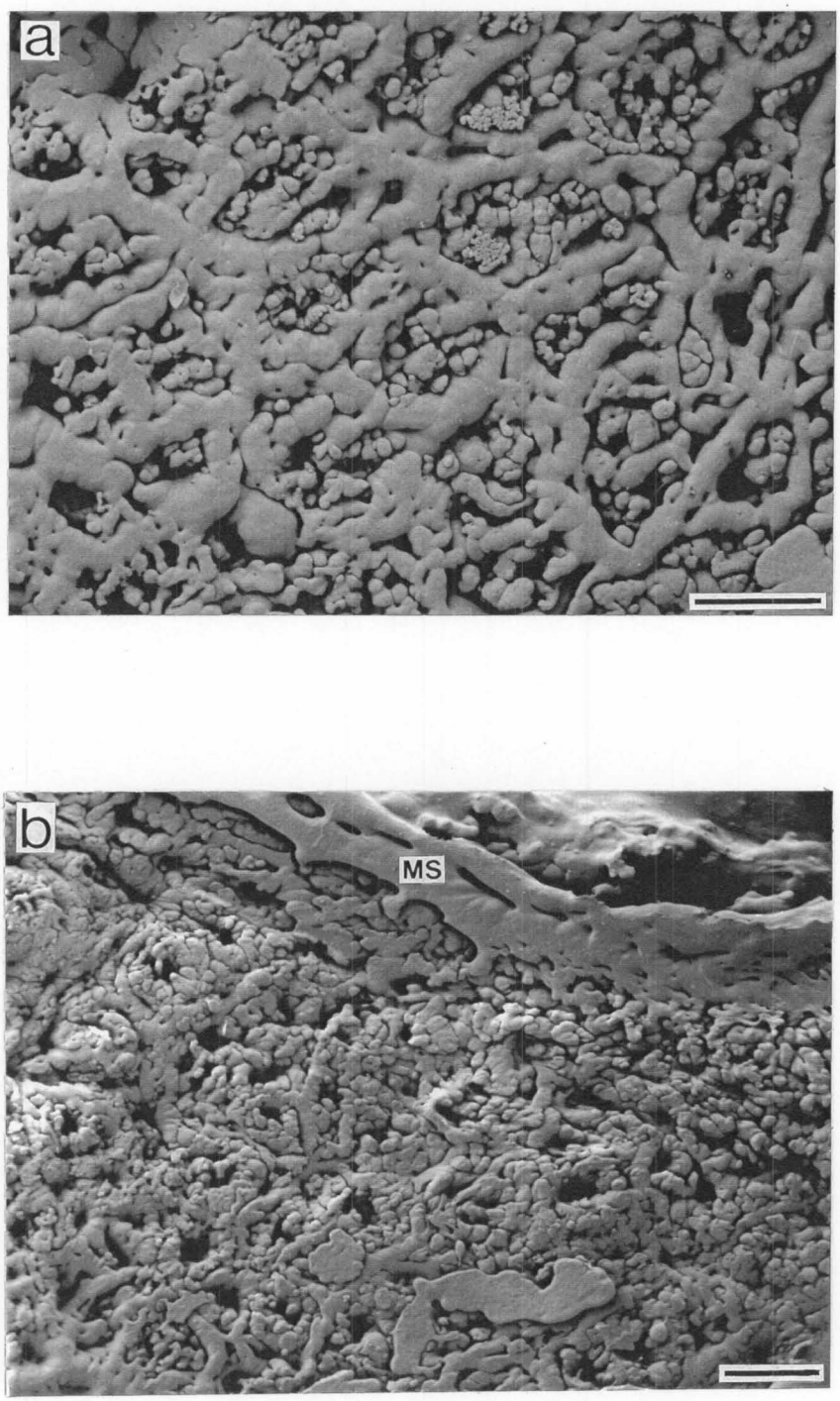

Fig. 4.9. a) A scanning electron micrograph of the ventral surface of a cast of the branchiostegal sinus as it is applied to the dorsal roof of the branchial chamber. Interconnecting rings of vessels can be seen with projecting lacunae between. b) Large marginal collecting sinuses (MS) border the posterior carapacial margin and collect haemolymph from the branchiostegite for return to the pericardium. Scale bars = 1 mm.

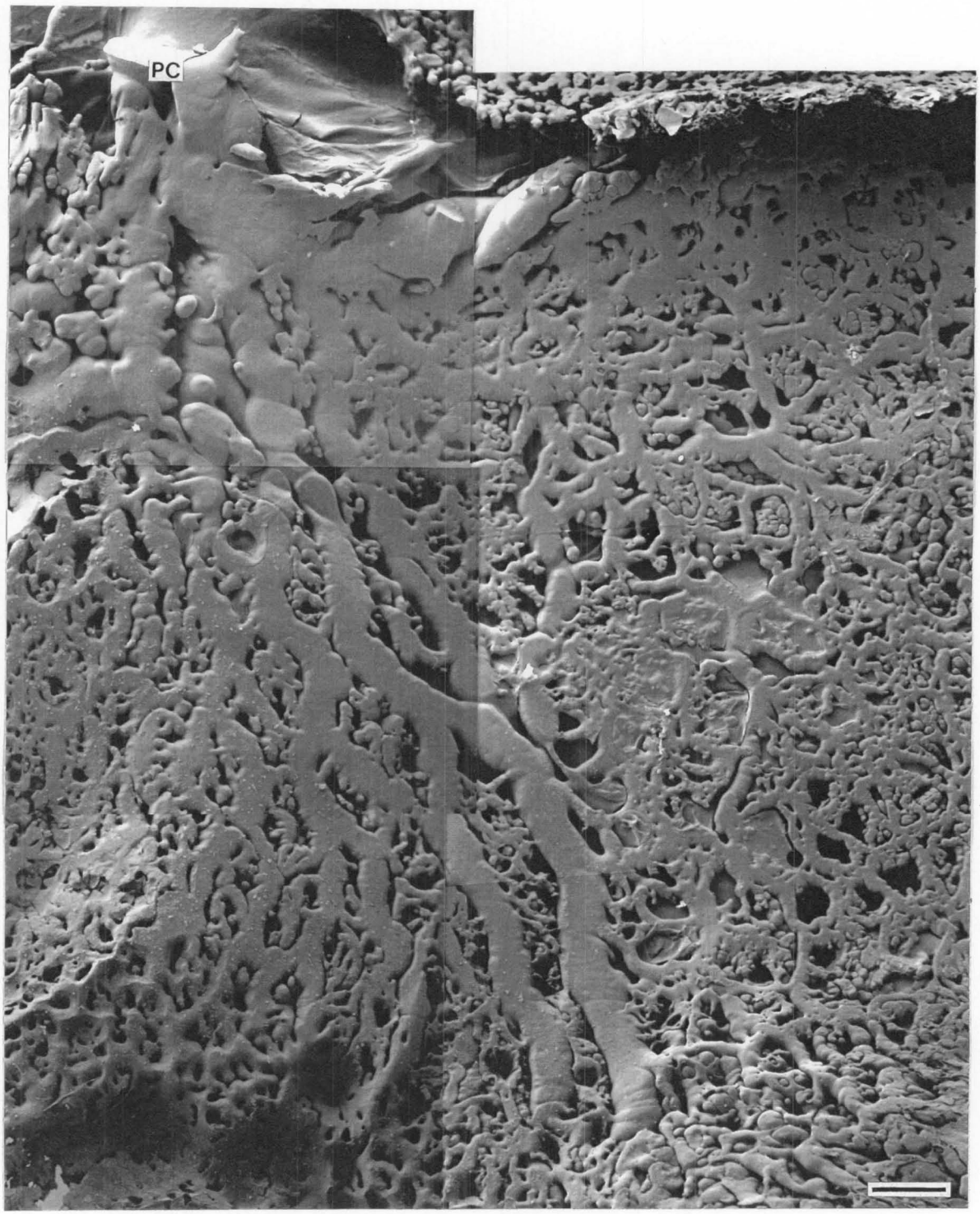

Fig. 4.10. A composite of several scanning electron micrographs of the ventral surface of a cast of the branchiostegal circulation showing the large collecting vessels which connect laterally to the pericardium at the point PC. Scale bar = 1 mm.

The branchiostegite and its associated vasculature have become greatly developed in terrestrial species, such as *Birgus latro* (pers. obs.), *Ocypode cordimanus* (Greenaway and Farrelly, 1990) and *Mictyris longicarpus* (Farrelly and Greenaway, 1987), to form a pulmonary system, superseding the gills as the primary sites for respiratory gas exchange. The arrangement of vessels in the lung is more highly organised than in the branchiostegites of aquatic species. But analogous structures can be seen, for example the terrestrial crab, *Geograpsus grayi* has large pulmonary veins running around the margin of the dorsal carapace, collecting haemolymph from the lungs and returning it posteriorly to the pericardium (Greenaway and Farrelly, 1990). These appear to be similar to the marginal collecting vessels seen in *O. catharus* and *C. novazealandiae*. In most terrestrial species the haemolymph is supplied to the respiratory surfaces via small lacunae (Greenaway and Farrelly, 1990). Similar blood spaces were also found in *O. catharus* (Fig. 4.9a). Thus it appears that the vascular origins of the terrestrial crustacean lung may be able to be traced back through semi-terrestrial species to ancestral-type fully aquatic species.

In aquatic species however, the main route for the return of haemolymph from the tissues to the pericardium, is via the gills, where oxygenation occurs. A longitudinal tubular sinus is situated at the bases of the limbs on each side, these are the infrabranchial sinuses. These sinuses are the collection vessels for the afferent supply of haemolymph to the gills. Anteriorly the infrabranchial sinus connects with the mass of small ventral sinuses in the head region, by the bases of the mouthparts. The pleural and leg sinuses drain into the infrabranchial sinus along the rest of its length.

O. catharus has nine pairs of phyllobranchiate gills. All nine pairs of gills receive haemolymph from the infrabranchial sinuses via the dorsal afferent branchial vessels. Oxygenation occurs as the haemolymph flows through the plate-like gill lamellae. Haemolymph leaving the lamellae then enters the ventral efferent branchial vessels. These drain into four large branchiopericardial veins situated superficially on the dorsal surfaces of the pleural muscle compartments. The walls of these vessels are formed by the endophragmal skeleton. The branchiopericardial veins take oxygenated blood from the gills to the pericardium. The efferent branchial vessels of gills 1 to 5 empty into the most anterior branchiopericardial vein. The second branchiopericardial vein arises as two smaller vessels which appear to be continuations of the efferent branchial veins of gills 6 and 7. The remaining two posterior gills, 8 and 9, supply the 3rd and 4th branchiopericardial veins, respectively.

Regulation of haemocoelic pressure and gill function

Recently much attention has been focused on the role of the dorsoventral muscles (DVM) in the regulation of haemocoelic pressure. Taylor and Taylor (1991) first showed that DVM activity in intact *Carcinus maenas*, was inversely correlated with changes in internal volume. Contraction of the DVM raises the flexible roof of the branchial chamber toward the dorsal carapace, compressing the large branchiostegal and hepatic sinuses lying between the two, this increases haemocoelic pressure. When the haemocoelic pressure was experimentally lowered by the removal of haemolymph, the activity of the DVM increased to restore internal pressure. Similar responses have also been demonstrated in *O. catharus* (Taylor et al., 1992) and in the semi-terrestrial species *Goniopsis cruentata* (Wilkens and Young, 1992).

Taylor (1990), using *O. catharus* and *C. maenas* gills, demonstrated that changes in gill transmural pressure (P_{trans} = internal gill pressure - external pressure) alter the vascular conductance of the gills *in-vitro*. Changes in P_{trans} can also occur spontaneously *in vivo* through fluctuations in branchial chamber pressure (P_{branch}) associated with ventilation. During forward ventilation, the sub-ambient branchial chamber pressures generated tend to favour perfusion of the gills by increasing P_{trans}. Alternately, ventilatory pauses and reversals, which produce relative increases P_{branch}, tend to reduce gill perfusion. Such effects would be more severe in a burrowing species like *O. catharus*, as fluctuations in P_{branch}, and thus P_{trans}, are much greater when buried (chapter 3). In unburied *O. catharus*, P_{branch} ranged from +3.64 cm H_2O to -4.6 cm H_2O over a range of ventilation volume ($\dot{V}_w$) from 0.95 l $kg^{-1}min^{-1}$ to 1.25 l $kg^{-1}min^{-1}$, in the reverse and forward ventilatory modes, respectively. Whereas in buried crabs, ventilating between 0.69 l $kg^{-1}min^{-1}$ and 0.51 l $kg^{-1}min^{-1}$, in the forward and reverse mode, respectively, P_{branch} varied between -9.69 and +4.16 cm H_2O. The positive branchial chamber pressures that accompany a ventilatory reversal could potentially compromise oxygen uptake by restricting the flow of haemolymph to the respiratory surfaces. In most brachyuran species reversals are very brief, lasting only a few seconds, and the effect of these events on respiration may be small. However many burrowing species, including *O. catharus*, show an increased dependence on the reverse ventilatory mode, especially when buried, thus the consequences of reverse ventilation for gill function may be more severe in these species, especially when buried.

Any change in gill volume, potentially resulting from changes in P_{branch}, will displace haemolymph into or out of the body, tending to elevate or depress haemocoelic pressure, respectively. In order to maintain a constant internal pressure

the haemolymph entering the body from the gills must be accommodated by an equal and opposite volume change at one or more of the other compliant body surfaces, such as the gut, arthrodial membranes or linings of the branchial chambers. The DVM could either act to maintain a constant gill P_{trans}, and gill perfusion, at the expense of a constant haemocoelic pressure, or *vice versa*. Rajashekhar and Wilkens (1991), also working on *C. maenas*, found that DVM activity decreased in response to both spontaneous and artificial increases in P_{branch} and *vice versa*. Presumably relaxation of the DVM allows haemolymph, displaced from the gills by the pulse of positive P_{branch}, to be accommodated in the branchiostegal sinus without elevating haemocoelic pressure, but also without restoring P_{trans}. Therefore, it appears that the DVM act to regulate haemocoelic pressure, while allowing gill P_{trans}, and potentially gill perfusion, to vary.

If gill function is compromised by increases in P_{branch} during ventilatory reversals, another facultative site of gas exchange may be utilised. The branchiostegites may provide such a site. Compression of the gills would result in the preferential perfusion of branchiostegites by the displaced haemolymph, due to relaxation of the DVM (Rajashekhar and Wilkens, 1991). Again this effect would be greatest in buried animals due to the higher recorded values of P_{branch}. This may help to explain why E_w% and $\dot{V}_w/\dot{M}o_2$ remained relatively high in buried reverse ventilating *O. catharus* when compared to unburied animals utilising this mode (Chapter 3). In addition, *O. catharus* shows strong epibranchial water flow during both forward and reverse ventilation (chapter 2). A large proportion of the inhalant water stream appears to pass between the dorsal surface of the gills and branchiostegal roof of the branchial chambers, especially during reverse ventilation. Hughes et al. (1969) noted a similar epibranchial flow in *Carcinus maenas* when utilising the forward ventilatory mode and they showed that this part of the water stream remained relatively well oxygenated. If this is also the case in *O. catharus*, then a high Po_2 gradient could be maintained across the branchiostegites which would favour the diffusion of O_2 into the haemolymph. This, of course, is subject to the capacity of the branchiostegal cuticle for the diffusion of oxygen. Preliminary examination of this area by transmission electron microscopy shows that the cuticle is comparatively thin, being in the order of 10 - 20 μm thick (R.N. Richards, pers comm.). While this is somewhat thicker than the cuticle of the gill lamellae in *O. catharus* (1 -2 μm) (Richards, 1992) some diffusion of gases may be possible allowing the branchiostegites to act as auxiliary gas exchangers. No value was given for branchiostegal cuticular thickness in *H. nudus* by Greenaway and Farrelly (1990).

Further work is required to examine the permeability of the branchiostegal cuticle and epithelium to O_2, before any solid conclusions can drawn regarding the

function of the branchiostegites as secondary gas exchange sites in *O. catharus*. Recordings of possible changes in haemolymph flow and Po_2 in the vessels of this region from buried and unburied animals utilising the two ventilatory modes would also be of great value.

Chapter 5

The Physiological Effects of Commercial Processes.

Abstract

Capture by potting and transport to shore-based holding tanks resulted in small perturbations of haemolymph acid-base balance. Haemolymph pH fell from 7.65 ± 0.03 to 7.44 ± 0.02 and [lactate] increased from 0.8 ± 0.4 to 4.02 ± 0.32 mmol l^{-1}. Recovery from these changes was rapid, with both variables being restored to settled values within 24 hours. This experiment highlights the benefits of careful handling of shellfish destined for sale in a whole live form and a number of recommendations for handling *O. catharus* are given.

Animals were experimentally emersed in order to simulate a period of air exposure, as would occur during live transportation. Following emersion, oxygen consumption ($\dot{M}o_2$) declined by 69% within 2 hours and remained stable at this level throughout the 12 hour emersion period. Haemolymph pH fell from 7.801 ± 0.080 to 7.351 ± 0.090 after 12 hours in air. The acidosis was at least partially metabolic in origin as haemolymph [lactate] increased from 0.6 ± 0.2 mmol l^{-1} to 8.7 ± 0.9 mmol l^{-1} during the same period. Elevated lactate levels suggest the accumulation of an oxygen debt during the emersion period. Evidence of repayment of this debt was seen as an increase in $\dot{M}o_2$ following reimmersion. Mean $\dot{M}o_2$ reached a maximum of 39.5 ± 3.2 μmol $kg^{-1}min^{-1}$ thirty minutes after the return to sea water. All variables were restored to pre-emersion levels within 12 hours. Despite large perturbations in haemolymph acid-base balance and a substantial reduction in $\dot{M}o_2$, mortality was low in animals that were left undisturbed during emersion (6%). Crabs that had blood samples removed during emersion showed a higher mortality (25%), presumably reflecting the increased stress due to blood loss and handling. These values are lower than mortalities previously reported for individuals of the red rock lobster *Jasus edwardsii* treated similarly. *J. edwardsii* is successfully exported live from New Zealand.

Monitoring of cardiac and ventilatory activity during emersion appears to provide a simple indicator of the condition of animals during emersion.

Introduction

In the last two decades the fishery based on *O. catharus* has grown rapidly, from 775 kg in 1977 to 306 000 kg in 1983 (Stead, 1984). Up until recently, virtually all of the catch was sold domestically as whole fresh, cooked or frozen crabs. However, there is increasing demand for live animals, which is an obvious assurance of product freshness. In response to this demand, crabs are now routinely transported live within New Zealand, either by road or by air freight. Because distances in this country are relatively short, animals are rarely emersed for periods longer than about 4 hours before reaching their destination.

It appears that there is considerable potential for further development of the Paddle crab fishery. With potential habitat, (ie. sandy beaches) making up 57% of the 13 000 km New Zealand shoreline, the maximum sustainable yield for this fishery may be as great as that for the New Zealand red rock lobster (*Jasus edwardsii*). However, there is only limited capacity for expansion of the domestic market. Therefore, for this potential to be realised much larger overseas markets must be developed. As with the domestic market, the major demand overseas is for live product.

To reach overseas destinations, crabs must be air freighted over much larger distances and often various stopovers are made en route. As a result, the aerial exposure time for the animals is increased to a minimum of 12-24 hours. This will present a major challenge to the respiratory system of a fully aquatic crab, such as *O. catharus*. The response of *O. catharus* to such a challenge has not yet been determined. Some trial international shipments have been made, but the survival of the crabs has been poor.

Therefore, a series of experiments were carried out to examine the respiratory and haemolymph acid-base changes that occur in *O. catharus* in response to actual and simulated commercial handling and live aerial transportation procedures. Specifically, the recovery from capture stress and the response to experimental emersion.

Heart rate and ventilation rate were also monitored in a group of crabs emersed until death occurred to see if these parameters could provide a simple technique for monitoring the condition of crabs during emersion.

Materials and methods

Changes in haemolymph pH and [lactate] during post capture recovery

Four circular steel and nylon mesh crab pots (1 m diameter) were baited with fish carcasses and set out randomly off Rabbit Island, Nelson in 3-4 metres of water. The pots were left overnight and were lifted the following morning. All four pots were lifted within 15 minutes of each other. A total of 84 crabs, of both sexes, ranging in size from 81 to 135 mm carapace width (approximately 105 g to 420 g), were caught.

Upon lifting and emptying the pots, the crabs were immediately transferred to an 80 l plastic trough filled with flowing sea water for the return trip to shore. As each pot was emptied, two crabs were randomly selected for haemolymph sampling. Using a 1 ml syringe fitted with a 20 g hypodermic needle, a 500 μl sample of prebranchial haemolymph was removed via the arthrodial membrane of the fifth pereiopod. The pH of these samples were immediately analysed using a Metrohm Herisau E488 pH meter and electrode, at ambient air temperature. A 100 µl subsample of haemolymph was added to 200 µl of ice cold 0.6% perchloric acid to deproteinate the sample. This was then frozen in dry ice and stored at -75°C to be used later for determination of haemolymph lactate concentration. A spectrophotometric enzymatic method was used to analyse the stored samples for lactate (Boehringer kit #139 084), with modifications suggested by Engel and Jones (1978). Changes in the absorbances of samples were read with a Kontron Uvikon 860 spectrophotometer.

Returning to shore took approximately 35 minutes. The trough was then drained and the crabs were transported overland in air to a holding tank. The crabs were thus emersed for 55 minutes before being placed in an 800 l circular black plastic tank filled with fresh aerated sea water at ambient temperature. Prebranchial haemolymph samples were taken from 10 randomly chosen crabs before reimmersion. The pH of the onshore samples was measured using a activon BJ332 flat bulb pH electrode thermostatted to the same temperature as the water in the holding tank, and connected to a Radiometer pHM84 research pH meter. Again a subsample of haemolymph was deproteinated and stored for subsequent analysis of lactate. This sampling procedure was repeated on 10 randomly chosen crabs at various time intervals throughout the recovery period.

The total weight of the crabs in the tank was 24 kg giving a loading of 0.3 kg

l^{-1} of water. Water in the holding tank was exchanged with fresh ambient sea water at a rate of 1500 l h^{-1} for approximately 4.5 hours centred on each high tide. The water in the tank was vigorously aerated at all times.

Experimental Emersion

Series I: Oxygen Uptake

Sixteen crabs of both sexes, weighing between 58 g and 521 g, were obtained from a local fisherman who fishes along New Brighton beach, Christchurch. The animals were brought to the Zoology Department of the University of Canterbury and maintained in a recirculating sea water system, at 15 ± 1°C, under a 12 h day (0830-2030)/12 h night light cycle, for at least 2 weeks before being used for experimentation. During this time the animals were fed twice weekly on freshly opened mussels (*Mytilus edulis* and *Perna canaliculus*). All animals were judged to be in the intermoult stage (C) of the moult cycle.

Aquatic oxygen consumption ($\dot{M}o_2$) was determined using closed box respirometry, while a constant pressure manometer system was used to calculate aerial $\dot{M}o_2$. Depending on their size, crabs were placed in either one of two sealable perspex boxes. A large rectangular box (volume = 1.8 l) was used for crabs weighing over 200 g and a small circular box (volume = 0.8 l) was used for smaller individuals. These were then submerged in filtered aerated sea water at 15 ± 1°C in a small recirculating sea water system (total volume approx. 600 l). All animals were allowed to settle in the respirometers for 12 hours before measurements began.

Both boxes were fitted with two 10 mm diameter ports, one on the lid and one on the side. These were connected with silicone tubing (Dow Corning silastic ID = 6.4 mm, OD = 9.5 mm) via small submersible pumps (Rena C40 and Rena C20, large and small respirometer, respectively).

At each sample time the boxes were sealed. One ml water samples were drawn, with replacement, from the sealed chambers via 1 ml hypodermic syringes fitted with 18 g hypodermic needles which were inserted through a rubber bung in the lid of each box. After a timed interval, a second sample was taken and the hose attached to the port on the lid was disconnected. This allowed the circulation of fresh aerated sea water through the boxes between samples. The pumps continued to run

while the respirometers were sealed, ensuring adequate mixing of the water within the boxes during aquatic $\dot{M}o_2$ determinations. The Po_2 of each water sample was determined using a Strathkelvin 1302 oxygen electrode, thermostatted to 15 ± 0.5°C, and a Strathkelvin 781 oxygen meter. By this means aquatic $\dot{M}o_2$ was measured at 6 hourly intervals for the first 12 hours to allow the animals to settle and for a further 24 hours to identify any daily rhythm that may exist.

After 36 hours in water the crabs were emersed, with minimal disturbance, by draining the water from the respirometers. Small mesh bags containing a CO_2 absorber (Sodasorb), were suspended from the bungs in the lids of the boxes. The air filled chambers were connected to manometers, sealed, and resubmerged to maintain constant temperature. The fall in volume in the respirometers, resulting from the uptake of O_2 by the crabs, was monitored using constant pressure manometry. Average $\dot{M}o_2$ was calculated over 1 and 2 hour periods for a total of 12 hours. Following emersion the sodasorb was removed and the respirometers were again filled with sea water. Measurements of aquatic $\dot{M}o_2$ were made at intervals during a further 24 hours to monitor recovery from emersion. Four runs were performed using empty respirometers as controls.

Series II: Haemolymph Acid-Base Status

12 large animals (> 310 g) were placed in plastic rectangular containers, with clear clip-on lids. These were submerged in fresh filtered sea water at 15°C in the same recirculating sea water system used in the respirometry experiment. Each box was supplied directly with a flow of aerated sea water to ensure that the animals did not experience hypoxia at any time. Eight of the animals were taken through the same periods of settlement, emersion and recovery as used in the emersion experiment. The remaining 4 animals stayed submerged for the entire period and served as controls for the effects of sampling. At each sample time, the boxes were lifted out of the sea water and opened. Using 1 ml syringes fitted with 20 g hypodermic needles, a 500 μl sample of haemolymph was withdrawn from the infrabranchial sinus via the arthrodial membranes of the fifth pereiopods. The lids were placed back on the boxes and they were resubmerged in the sea water. The pH of these samples was measured at 15°C using a G297 pH electrode and Radiometer pHM84 research pH meter. A 100 μl subsample of the haemolymph was deproteinated with the addition of 200 μl of ice cold perchloric acid, frozen in liquid N_2, and stored at -75°C. These subsamples were analysed for lactate using a YSI model 23L lactate analyser.

Prebranchial haemolymph samples were also taken randomly from 10 of 15 crabs air freighted from Nelson to Christchurch. The samples were taken in the same manner as described above and stored at -75°C before being analysed spectrophotometrically (Boehringer kit #139 084, with modifications suggested by Engel and Jones (1978)). Changes in the absorbances of samples were read with a Kontron Uvikon 860 spectrophotometer.

Heart and scaphognathite activity in emersed crabs

Nine crabs, weighing between 154 and 300 g were used. The left ventilation rate (LF_r) of each animal was monitored by impedance techniques. Small silver wire impedance electrodes were inserted though holes drilled in the carapace on either side of the left scaphognathite. The electrodes were held in place using rubber dam and cyanoacrylate cement. Heart rate (F_h) was also monitored via impedance electrodes inserted through small holes drilled through the dorsal carapace on either side of the heart. The crabs were returned to sea water (15°C) and left undisturbed overnight to recover from the operation. The next day the electrodes were connected to a 2 channel Bioscience A100 impedance coupler the signals from this were amplified by Gould 13-4615-58 universal amplifiers and the output displayed and recorded on a Gould 8188-2202-XX two channel thermal writing recorder. Initial LF_r and F_h were recorded from the settled immersed crabs before they were emersed. The crabs were removed from the water by hand and placed in 17 l clear perspex aquaria where they were held in air at room temperature (20 - 22°C) until all ventilatory and cardiac activity had ceased. During this time F_h and LF_r were monitored at approximately thirty minute intervals.

Analysis of data

All data are presented as the mean ± one standard error of the mean. All unpaired means were compared by one-way ANOVA with significant differences between means being identified by using Duncan's new multiple range test. Significance was determined at a 5% probability level.

Results

Haemolymph pH and [lactate] during Post capture recovery

Fig. 5.1 shows changes in haemolymph pH and [lactate] after capture by potting. The mean haemolymph pH immediately following lifting and emptying of the pots was 7.65 ± 0.03. pH had fallen significantly to 7.44 ± 0.02 by the time the crabs had reached the onshore holding tank (Duncan's New Multiple Range test, $\alpha = 0.05$) and continued to decline until a minimum of 7.20 ± 0.02 was reached 2 hours after the crabs had been introduced to the holding tank. After this time haemolymph pH began to rise. After between 16 and 24 hours in the holding tank pH had returned to a value not significantly different to that seen following the lifting of the pots. After 24 hours recovery, haemolymph pH was significantly higher than the initial value at 7.72 ± 0.02 (Duncan's New Multiple Range test, $\alpha = 0.05$). There was a second small drop in pH during the next 8 hours to a minimum of 7.57 ± 0.02. This value was significantly lower than both the initial value (Duncan's New Multiple Range test, $\alpha = 0.05$). However by the end of the 48 hour recovery period pH had risen again to 7.75 ± 0.02, which was not different to the 24 hour value but was significantly higher than the initial value (Duncan's New Multiple Range test, $\alpha = 0.05$).

A small significant increase in haemolymph [lactate] accompanied the acidosis (Duncan's New Multiple Range test, $\alpha = 0.05$) (Fig. 5.1). Initially, when the pots were lifted, the haemolymph [lactate] was very low at 0.8 ± 0.4 mmol l^{-1}. A peak concentration of 4.02 ± 0.32 mmol l^{-1} was reached at the time the animals were placed in the holding tank. The maximum individual haemolymph [lactate] of 8.6 mmol l^{-1} was also recorded at this time. Haemolymph [lactate] was restored to the initially recorded level within 2 hours (Duncan's New Multiple Range test, $\alpha = 0.05$). Haemolymph [lactate] did not vary significantly from the initial value for the remainder of the experimental period. However a second small increase in [lactate] was seen after 16 hours in the holding tank, reaching 1.19 ± 0.27 mmol l^{-1}. This was significantly higher than the two adjacent values (Duncan's New Multiple Range test, $\alpha = 0.05$).

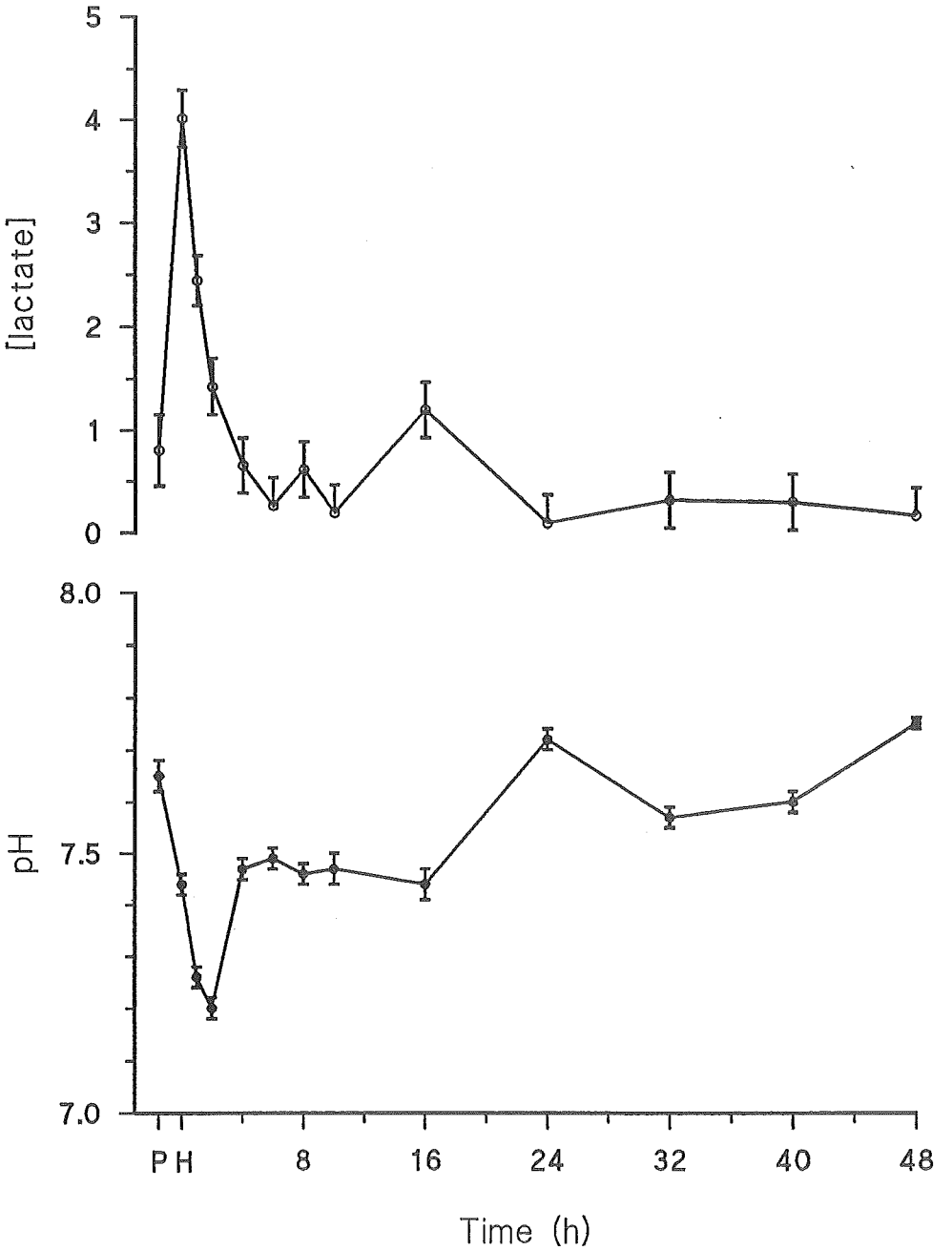

Fig. 5.1. Changes in haemolymph pH and [lactate] (mmol l^{-1}) in *O. catharus* following capture by potting. P is the sample taken immediately following lifting and emptying of the crab pots, and H = 0 hours and is the sample time immediately prior to the animals being released into an onshore holding tank for recovery. n = 8 for P and n = 10 for all other samples. Data are shown as mean ± 1 s.e.m.

Experimental emersion

Series I: oxygen uptake

During the 24 hours preceding emersion $\dot{M}o_2$ fell from 24.7 ± 3.2 μmol kg^{-1} min^{-1} (n = 16) at noon to 16.5 ± 3.2 μmol $kg^{-1}min^{-1}$ (n = 16) by midnight and had returned to 23.0 ± 3.2 μmol $kg^{-1}min^{-1}$ (n = 16) at noon the following day (Fig. 5.2). This latter value was that recorded immediately prior to emersion. None of these differences were significant (α = 0.05). It was assumed that these changes indicated circadian fluctuations in $\dot{M}o_2$. Where appropriate these were used as control values against which the $\dot{M}o_2$ emersion during and recovery could be compared.

A reliable value of $\dot{M}o_2$ could not be obtained for the first hour of emersion while the respirometers were being drained of water, resubmerged and connected to the manometers. The first value of $\dot{M}o_2$ in air is given for the period between 1 and 2 hours following emersion, this was 7.1 ± 0.8 μmol $kg^{-1}min^{-1}$ (n = 8). This value was significantly lower than the aquatic $\dot{M}o_2$ recorded immediately prior to emersion (α = 0.05). $\dot{M}o_2$ remained essentially constant during the emersion period. Between 10 and 12 hours after emersion mean $\dot{M}o_2$ was 6.1 ± 0.7 μmol $kg^{-1}min^{-1}$ (n = 11). One crab of the 16 died during the emersion period (= 6% mortality).

The animals were reimmersed in sea water at midnight. Thirty minutes after reimmersion $\dot{M}o_2$ had increased greatly to 39.5 ± 3.2 μmol $kg^{-1}min^{-1}$ (n = 13). This value was significantly higher than the aquatic $\dot{M}o_2$ recorded at midnight prior to emersion (α = 0.05). $\dot{M}o_2$ declined rapidly over the next 1.5 hours and by this time was not significantly different to the nearest pre emersion $\dot{M}o_2$ value which had been recorded the previous midnight (α = 0.05). Eight hours after reimmersion $\dot{M}o_2$ had fallen to 21.6 ± 3.0 μmol $kg^{-1}min^{-1}$ (n = 15), then began to rise again to 25.4 ± 2.8 μmol $kg^{-1}min^{-1}$ (n = 15) at 12 hours following reimmersion (midday). Then $\dot{M}o_2$ began to decline and continued to do so until the end of the experiment (at midnight) finishing at 14.0 ± 1.7 μmol $kg^{-1}min^{-1}$ (n = 15). This value was not significantly different to that recorded at midnight prior to emersion (α = 0.05).

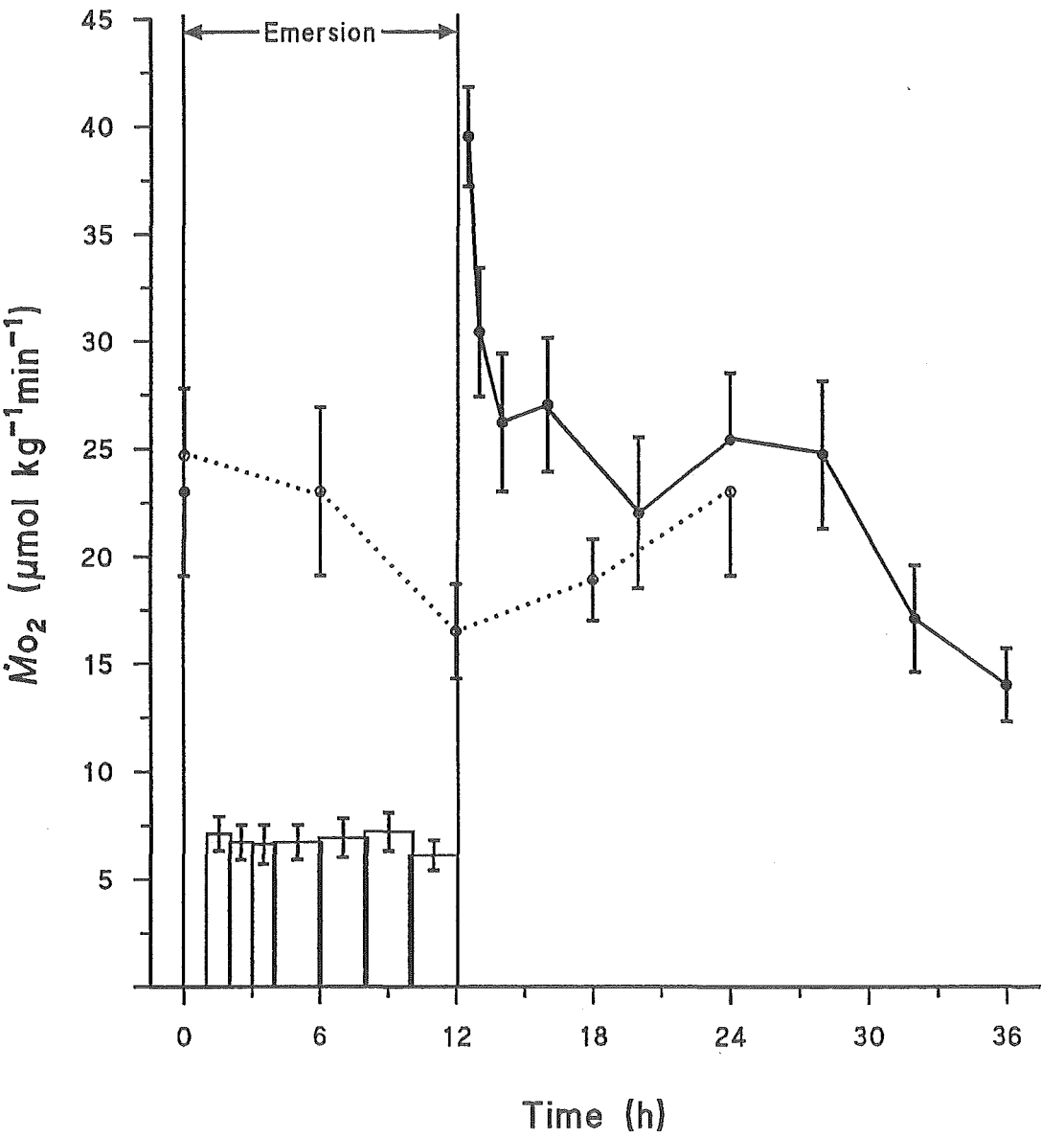

Fig. 5.2. Changes in $\dot{M}o_2$ in *O. catharus* at 15°C, resulting from a 12 hour period of emersion in air. O- - -O depicts aquatic $\dot{M}o_2$ during the 24 hours prior to emersion. This has been overlain on the graph to indicate the predicted $\dot{M}o_2$ during the emersion period. Note that the last point on this line is the same as the aquatic $\dot{M}o_2$ recorded immediately prior to emersion. Bars are used during emersion as each value is the average $\dot{M}o_2$ recorded over a 0.5, 1 or 2 hour period. Data are presented as mean ± 1 s.e.m.

As animals of a range of sizes were used in this experiment, the relationship between weight specific $\dot{M}o_2$ and mass could be determined (Fig. 5.3). This relationship was described by the least squares regression equation log(y) = -0.4284log(x) + 0.8097, $r^2 = 0.3429$. The slope of this line was significant ($t = -2.5036$, $p < 0.05$). The aquatic $\dot{M}o_2$ values obtained from the 14 crabs settled for 24 hours following emersion were used for this calculation.

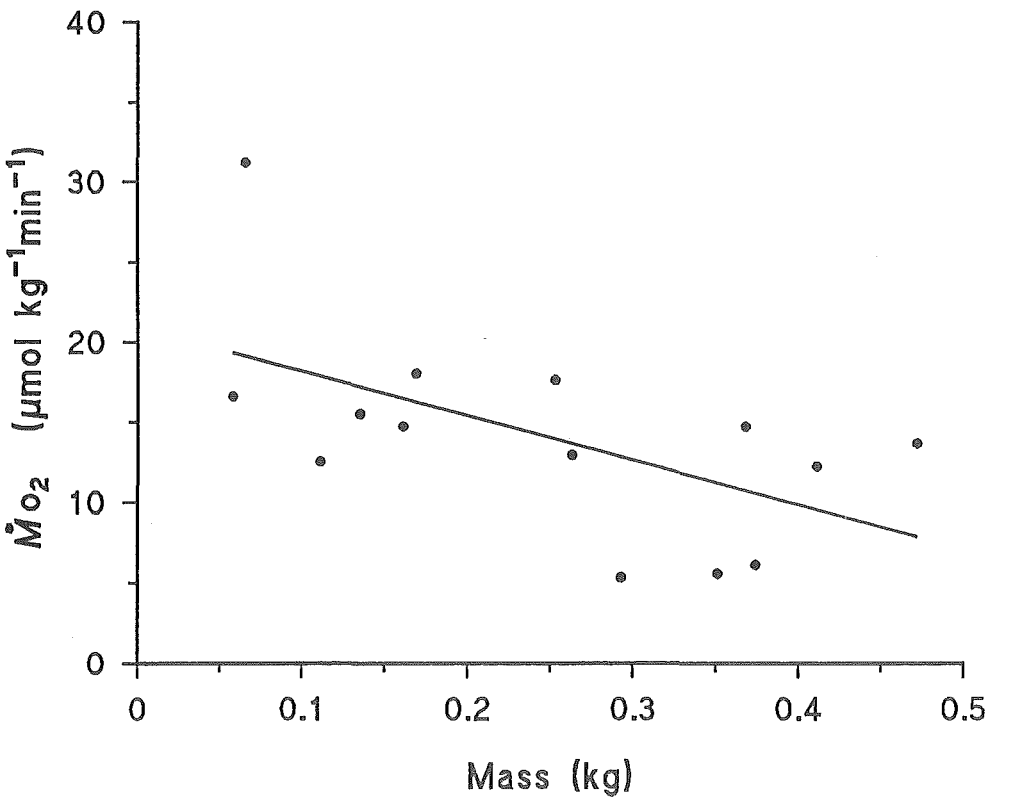

Fig. 5.3. Plot of log $\dot{M}o_2$ on log weight for *O. catharus* allowed to settle for 24 hours following emersion. The line indicates the regression equation for the significant relationship between the two, calculated by the least squares method to be log(y) = -0.4284log(x) + 0.8097.

Series II: haemolymph acid-base status

Prior to emersion, haemolymph pH was 7.801 ± 0.080 (n = 8) and haemolymph [lactate] was very low at 0.6 ± 0.2 mmol l^{-1} (n = 8). Upon emersion the pH began to fall and was significantly different from the pre emersion value between 6 and 12 hours into the emersion period (α = 0.05). After 12 hours of emersion pH had fallen to 7.351 ± 0.09 (n = 7). Haemolymph [lactate] had increased significantly to 3.2 ± 0.6 mmol l^{-1} (n = 8) (α = 0.05) after 6 hours in air and continued to rise throughout the emersion period, reaching a peak value of 8.7 ± 0.9 mmol l^{-1} (n = 8) after 12 hours in air.

With reimmersion, pH increased rapidly and was restored to pre emersion levels within 4 hours following the return to seawater (α = 0.05). The pH remained constant until the end of the experiment. Haemolymph [lactate] fell sharply following reimmersion, reaching preimmersion levels after 4 to 12 hours in water (α = 0.05). Haemolymph pH and [lactate] values in the control animals did not differ significantly from those recorded from well settled animals prior to emersion (α = 0.05). Thus the repetitive sampling used did not have a significant effect. Two of the 8 crabs died during the experiment giving a mortality of 25%. One died between 6 and 12 hours in air, and the other died within 4 hours of reimmersion. This higher mortality presumably reflects the added stress imposed by haemolymph sampling.

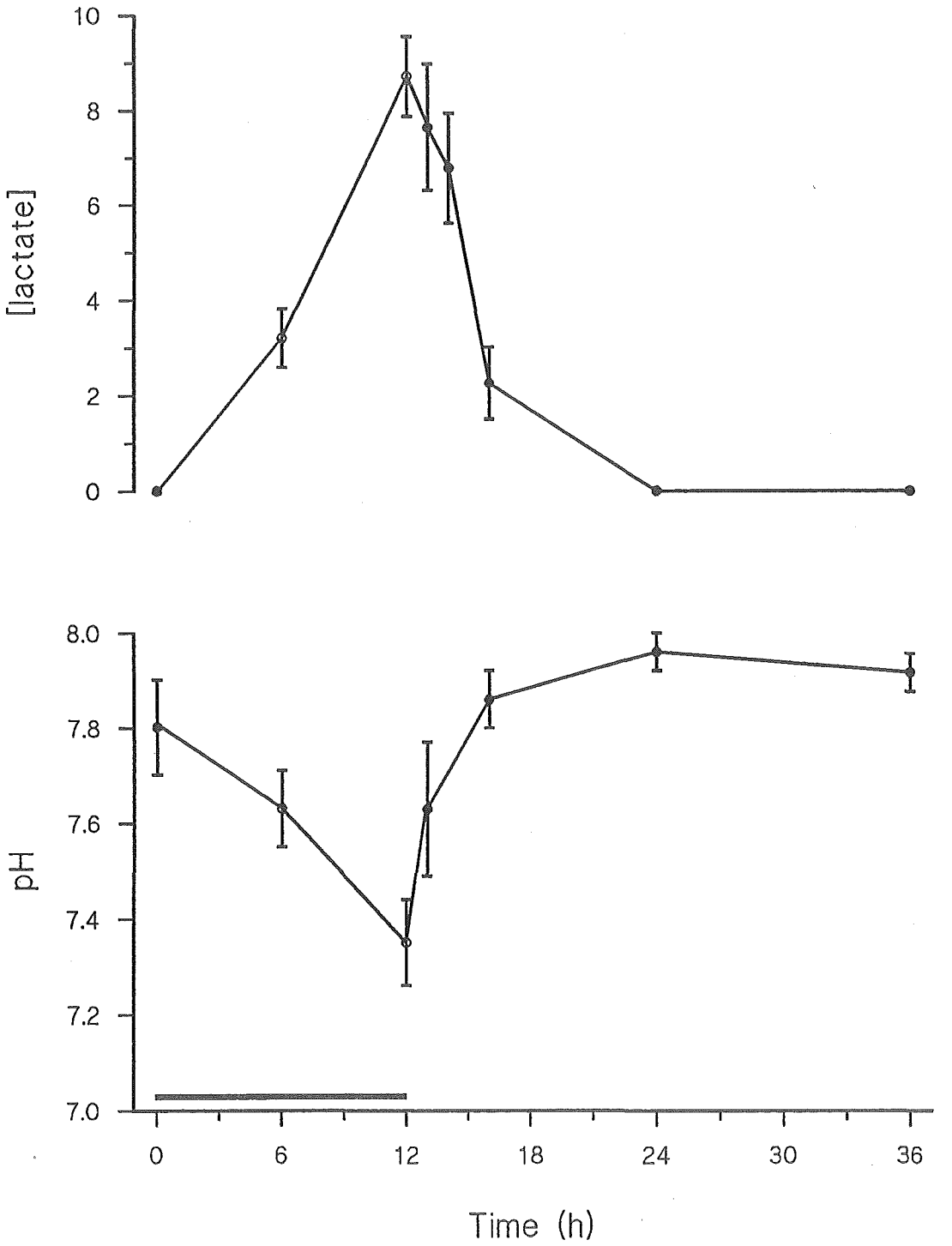

Fig. 5.4. Changes in haemolymph pH and [lactate] (mmol l^{-1}) in *O. catharus* during and following a 12 hour period of emersion at 15°C. ○———○ are values during emersion, ●———● are values during immersion prior to, and following emersion. The values at time = 0 are the pre-emersion values for the variables taken from immersed animals allowed to settle for 24 hours. The horizontal bar indicates the emersion period. Data presented as mean ± 1 s.e.m. (n = 8).

In addition to this controlled emersion experiment, haemolymph samples were taken from 15 crabs that were air freighted from Nelson to Christchurch. The crabs were packed vertically, with the mouthparts facing upwards, in a large polystyrene box at ambient temperature. Holes were made in two diagonally opposite corners of the box to allow ventilation. Due to delays these crabs were out of water for approximately 5 hours. Blood samples were taken randomly from 10 of the 15 animals prior to reimmersion. The mean haemolymph [lactate] was 6.4 ± 0.9 mmol l^{-1} with the individual minimum and maximum being 2.7 mmol l^{-1} and 11.9 mmol l^{-1}, respectively. The mean pH was 7.29 ± 0.08 with a range of 7.16 to 7.41. The condition of the crabs upon reimmersion could be described as fair, with most animals struggling weakly when handled. One of the fifteen crabs died within a day of being returned to sea water.

Heart and scaphognathite activity

At 20 - 22°C, the mean time to ventilatory failure in air was 15.9 ± 2.2 hours (n = 8) while the mean time to cardiac failure was 24.7 ± 2.6 hours (n = 8). In all crabs ventilation ceased before the heart. Time to cardiac failure ranged from 15 to 35 hours. Because of this variability all results are expressed in terms of a percentage of the time taken for cardiac activity to cease. Fig. 5.4 shows changes in left ventilation rate (LF_r) and heart rate (F_h) during the emersion period. Prior to emersion mean LF_r was 67.7 ± 6.9 beats min_{-1} and F_h was 48.5 ± 6.0 beats min^{-1}. When the crabs were initially placed in air both LF_r and F_h increased sharply to 131.4 ± 21.3 beats min^{-1} and 84.6 ± 12.5, respectively. This was probably largely due to the spontaneous activity and handling stress that accompanied emersion. After this, the animals quickly became quiescent and a rapid reduction was seen in both LF_r and F_h. As the emersion progressed the animals appeared to become moribund, with no movement of appendages, the antennae flattened and the eyestalks retracted into the orbits. However, this was not the case as the animals were still very responsive to visual, vibrational and tactile stimuli. As ventilation began to fail, the third maxillipeds began to hang down and the legs became limp. No response could be elicited using tactile, or other, stimuli. From outward appearances the animals appeared dead at this stage even the though the heart continued to beat. There was no change in the appearance of the animals until cardiac activity ceased. Mean LF_r had reached zero by 80% of the total time taken for mean F_h to do the same. Four crabs were placed back in sea water immediately following cessation of ventilation to see if any recovery occurred, none of these animals recovered. No transient pauses in LF_r or F_h were seen during the experiment.

back in sea water immediately following cessation of ventilation to see if any recovery occurred, none of these animals recovered. No transient pauses in LF_r or F_h were seen during the experiment.

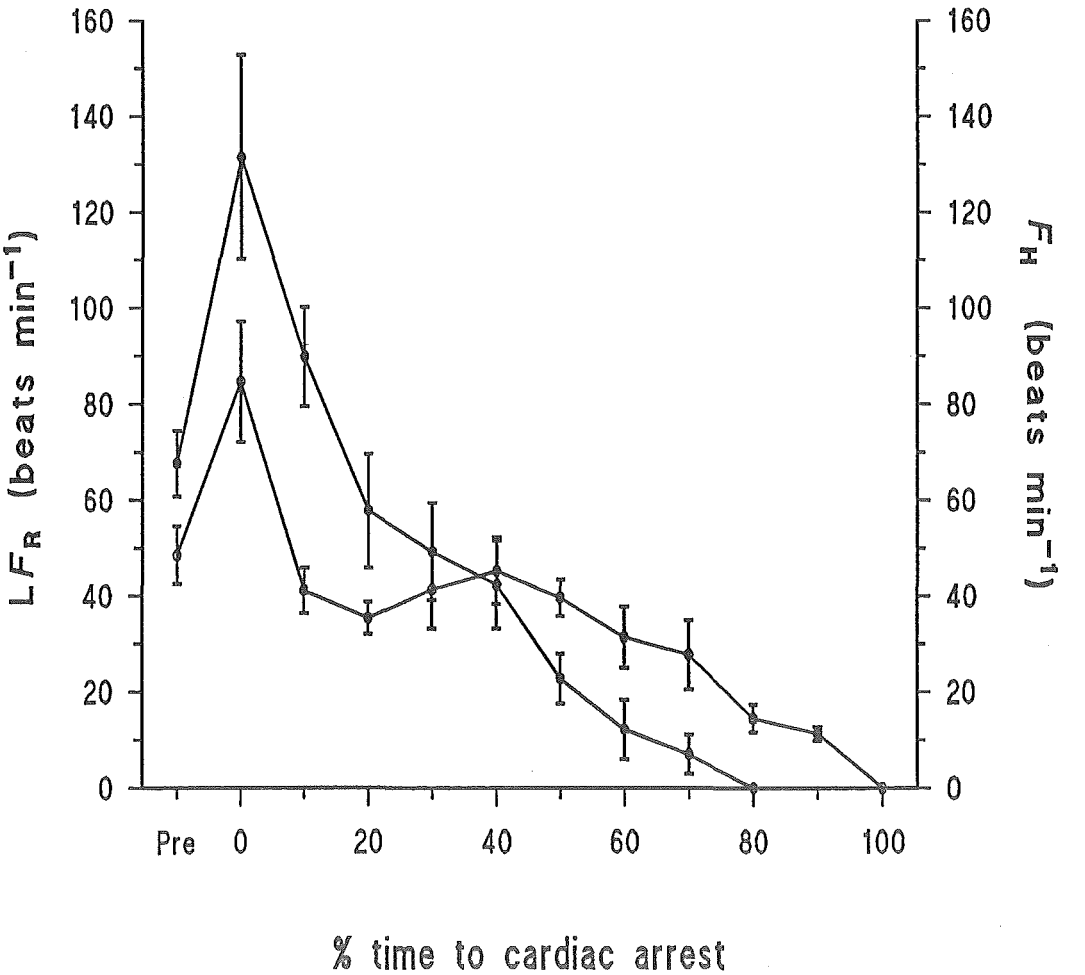

Fig. 5.5. Changes in cardiac frequency (F_h, ○) and left ventilation frequency (LF_r, ●) in emersed *O. catharus* at 20 - 22°C. Both variables are plotted against the percentage of the total time taken for the

Discussion

Post capture recovery

The observed post capture acid-base response was essentially a combined exercise and emersion response. The acidification of the haemolymph that occurred had a metabolic component, indicated by the increase in circulating levels of metabolic acids, and also probably a respiratory component, resulting from increased levels of carbon dioxide. When in air gill function is impeded limiting both oxygen uptake (see below) and CO_2 excretion across the gills. Haemolymph pH fell from 7.65 ± 0.04 to 7.21 ± 0.03, which represents nearly a tripling of the $[H^+]$ in the haemolymph. By comparison, 15 minutes of vigorous exercise by swimming resulted in a smaller drop in haemolymph pH from 7.63 ± 0.07 to 7.47 ± 0.06, approximately a 1.5 fold increase in $[H^+]$ (Chapter 2). Accumulation of CO_2 would probably not be as great in the exercised animals as they remained immersed throughout the exercise period. CO_2 is very soluble in water and can be easily lost across the gills to the external medium. The metabolic acid load produced during swimming was also smaller than that in the post capture animals. Haemolymph [lactate] from the post capture animals was more than twice as high as that following exercise alone (4.02 ± 0.79 mmol l^{-1} and 1.48 ± 0.34 mmol l^{-1}, respectively). Again these differences probably reflect the additional stress imposed by the period of emersion following capture.

Typically disturbances in haemolymph pH are restored more rapidly than [lactate] (Wood and Randall, 1981). Much of the proton load can simply be exported across the permeable body surfaces, such as the gills, and dumped into the surrounding well buffered sea water. Lactate is a large molecule and is not lost to the environment, but is retained and slowly converted back to either glucose or glycogen via gluconeogenesis (Booth et al., 1982; McDonald et al, 1979; Phillips et al., 1977; Whitely and Taylor, 1992). However, following capture, lactate levels were restored quickly, within 2 hours of being immersed in the holding tank, while pH reached its minimum value at this time and took a further 14 to 22 hours to return to the initial value. The crabs were active once they were reimmersed and it is possible that this activity may have depressed haemolymph pH through the formation of a respiratory acidosis. This activity probably would not have been vigorous enough to require the utilisation of anaerobic metabolism, thus additional lactate would not be produced.

Following recovery from the initial haemolymph acidosis continuing small fluctuations were seen in pH. These appear to be related to time of the day,

suggesting a daily rhythm. In Fig. 5.6 periods of low pH coincide with the hours of darkness, while the periods of high pH coincide with the daylight hours. *O. catharus* is nocturnally active coinciding with the periods of low pH, supporting the suggestion that spontaneous activity may serve to lower haemolymph pH. Haemolymph [lactate] remained relatively unchanged over this period. These findings may explain the apparent prolonged depression of haemolymph pH following capture.

The data suggest that a recovery period of 24 hours would be sufficient to allow full restoration of haemolymph pH and [lactate] following capture, before subjecting the animals to further handling and stress, such as transportation to market. Anecdotal accounts from fishermen suggest a longer holding period of 2-3 days allows fewer mortalities during subsequent emersion. This requires further investigation.

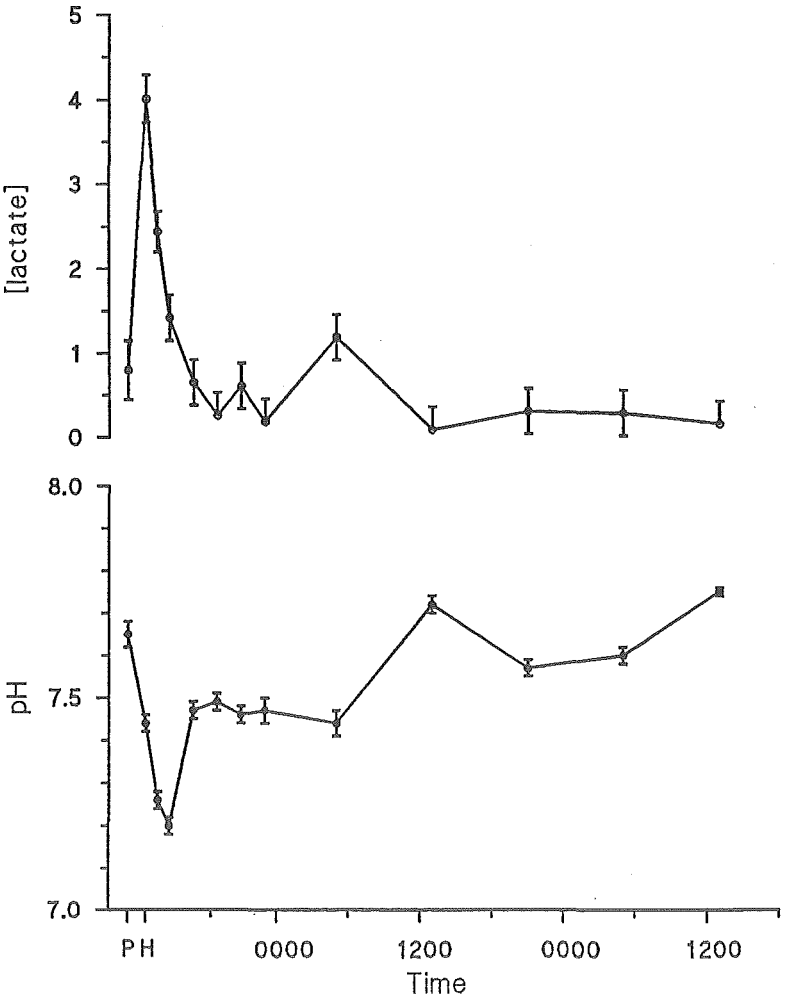

Fig. 5.6. Changes in haemolymph pH and [lactate] (mmol l^{-1}) following capture by commercial potting plotted against the time of day. This information is presented in Fig. 5.1 plotted against cumulative time. See Fig. 5.1 for an explanation of symbols.

The animals used in this experiment would have been subjected to a similar degree of handling to crabs fished commercially. Typically in a commercial operation, after the pots are lifted, the crabs must be sorted according to size and also perhaps sex. Fishermen selling live crabs only, usually return all animals smaller than about 250 g (approx 105 mm carapace width), if the fisherman is also processing crabs for extraction of the meat, then animals down to about 200 g (100 mm carapace width) are also taken. Depending on the fishermans individual policy, females, regardless of whether they are carrying eggs or not, are returned. Sorting involves handling the animals, and often the deck area is limited resulting in the animals fighting amongst themselves. This the elevates metabolism, intensifying changes in haemolymph pH and [lactate]. Usually animals destined to be sold live are handled more carefully, as the loss of limbs and other injuries is undesirable. The experimental animals were tipped out of the pots and onto the deck of the boat, with no special care being taken to reduce handling stress. In addition, when the animals arrived at the holding facility they were handled in order to be measured, this would equate to the stress imposed by handling the animals during sorting out at sea in a commercial operation.

The major difference between the experimental procedure and a commercial operation probably lies in the time the animals would spend out of water. The experimental animals were immersed during the return to shore, and were then emersed for only 55 minutes. In a commercial operation the elapsed time from when animals are landed on a boat to when they are placed in holding tanks would be considerably longer as a much greater number of pots would have to lifted before returning to shore. Usually crabs are not reimmersed until the holding facility is reached, and while some commercial fisherman take no action to prevent desiccation, many cover the animals with wet sackcloth or some other material. In the stone crab *Menippe mercenaria* this method combined with regular wetting, is as effective for reducing mortality, due to desiccation, as complete immersion (Simonsen and Hochberg, 1986). Nonetheless, greater perturbations in blood chemistry are likely to occur as the result of the longer emersion times and it is likely that the greater changes in haemolymph chemistry that may occur during a true commercial operation, would require longer recovery times.

Interestingly, despite their aggressive nature, *O. catharus* are very tolerant of being held at high densities. When emersed there is a tendency for the animals to hold and crush any object within reach with the powerful chelae. This results in a lot of damage to the animals, most commonly limbs are crushed or lost. But when immersed this tendency disappears, and instead the animals tend to fend each other off with the chelae, without nipping. Many of the damaged animals will die from their wounds. Usually this does not happen immediately, instead the animals may perish

in the holding tanks, fouling the system. In those which survive the wounds tend to quickly become necrotic, developing blackened margins. Such injuries make many crabs unsuitable for sale in a whole live or cooked form. Instead these animals must be either processed for the extraction of meat, if the facilities are available, or discarded. Thus immersing the animals during transportation to shore appears to have additional benefits, especially for animals destined for the live market.

This experiment shows *O. catharus* can be caught by commercial methods with minimal stress, and that by careful handling, animals can be returned to shore-based holding facilities in excellent condition.

Experimental emersion

O. catharus showed a relatively large (69%) reduction in $\dot{M}o_2$ from 23.0 ± 3.2 μmol $kg^{-1}min^{-1}$ prior to emersion, to 7.1 ± 0.8 μmol $kg^{-1}min^{-1}$ within 2 hours of being emersed. This is a typical response of many aquatic crustaceans to emersion. By comparison, *Pachygrapsus crassipes* showed a 55% reduction in $\dot{M}o_2$ with emersion (Burnett and McMahon, 1987). In *Callinectes sapidus*, a species closely related to *O. catharus*, $\dot{M}o_2$ fell by 35% when emersed (O'Mahoney and Full, 1984), and in *Liocarcinus puber*, $\dot{M}o_2$ was reduced by 81% (Johnson and Uglow, 1985). Aquatic brachyura have delicate phyllobranchiate gills, made up of successive layers of flat plate-like lamellae. When brachyurans are brought into air the gill lamellae clump together, due to surface tension effects of water trapped between the lamellae (deFur et al., 1988). This reduces the available surface area for gas exchange imposing a severe diffusion limitation on the respiratory system. Lamellar clumping may also increase the resistance to intralamellar haemolymph flow, limiting perfusion of the gills (deFur and McMahon, 1984). As mentioned previously, excretion of CO_2 across the gills is affected similarly. In *O. catharus*, $\dot{M}o_2$ was virtually unchanged throughout a 12 hour emersion period, indicating that the function of the gills remained inhibited.

Waldron (1991) demonstrated a 56% reduction in $\dot{M}o_2$ in the Macruran rock lobster *Jasus edwardsii*, following emersion. *J. edwardsii* is the only crustacean species that is currently exported live from New Zealand. Waldron (1991) has done a number of experiments using this species that are very similar to those in the present study. Since *J. edwardsii* is able to survive a transportation process that *O. catharus* would also have to endure, Waldrons work provides a useful comparison against which the responses of *O. catharus* can be compared.

After 12 hours in air, the mean haemolymph pH in *O. catharus* had fallen from

7.801 ± 0.080 to 7.351 ± 0.082. Acidification of the haemolymph is a typical crustacean response to emersion, for example the shore crab *Carcinus maenas* showed a drop in haemolymph pH from 7.824 ± 0.008 to 7.594 ± 0.018 after 9 hours in air (Truchot, 1975) and after 18 hours in air, haemolymph pH in the crayfish *Austropotamobius pallipes* had fallen from 7.896 to 7.452. Changes in haemolymph pH in *J. edwardsii* during an 8 hour emersion period (7.669 ± 0.019 to 7.343 ± 0.031, Waldron (1991)) were similar to those recorded from *O. catharus*. However, without knowing the buffering capacity of the haemolymph of *O. catharus*, a meaningful comparison of these results is difficult. The continual fall in pH observed in *O. catharus* during emersion suggests that, like *J. edwardsii*, this species has a poor ability to compensate for the increasing proton load.

The pH changes in *Ovalipes catharus* at least partially indicate a metabolic acidosis as mean haemolymph [lactate] increased from 0.6 ± 0.2 to 8.7 ± 0.9 mmol l^{-1} during 12 hours of emersion. The rate of accumulation of lactate in the haemolymph in *Ovalipes catharus* appeared to be similar to that seen in emersed *J. edwardsii* which showed a mean [lactate] of 4.2 ± 0.8 mmol l^{-1} after 8 hours in air (Waldron, 1991). This is perhaps surprising given the greater reduction in $\dot{M}o_2$ seen in *O. catharus* upon emersion, one might expect a greater dependence on anaerobic metabolism in this species, resulting in a greater accumulation of anaerobic end products. However, it is possible that the decline in $\dot{M}o_2$ in emersed *O. catharus* may, at least partially, represent a fall in the oxygen requirements of the animal, and thus the contribution of anaerobic metabolism. It is also possible that levels of lactate in the haemolymph do not accurately reflect those in the intracellular fluid. Substantial muscle:plasma [lactate] ratios have been found in several elasmobranch fish species following exercise, with tissue levels up to 760 times greater than those found in the plasma (Davidson, 1988). Phillips et al. (1977) have suggested that high tissue to blood [lactate] gradients may also occur in crustaceans, and the true contribution of anaerobic processes to energy production may be underestimated by the observed haemolymph levels.

Like *J. edwardsii*, the rate of appearance of lactate in the haemolymph in *O. catharus* increased with increasing emersion time. This is likely to be due to a lag between the formation of lactate in the tissues and its appearance in the haemolymph resulting in increased tissue:haemolymph [lactate] gradients over time. Similarly, in the elasmobranch *Squalus acanthias* following exhaustive exercise, the greatest tissue to plasma lactate gradients coincided with the highest plasma [lactate] values (Davidson, 1988). In both bony and cartilaginous fishes, benthic forms tend to show a high degree of metabolism of lactate *in situ* in the muscle, without releasing it to the haemolymph (Davidson, 1988; Milligan and McDonald, 1988). Despite being

periodically highly active, the burrowing habit of *O. catharus* gives this species a similar niche to benthic fish and it is possible that *O. catharus* also exhibits non-release of lactate from the muscle. Booth and McMahon (1985) estimated that as much as 56% of the total lactate load accumulated by *C. sapidus* during 30 minutes of swimming was released into the haemolymph. This is much greater than in vertebrates which typically release only about 10% of the total lactate load accumulated in the skeletal muscles during exercise into the bloodstream.

Recovery of haemolymph pH and [lactate] following reimmersion in *O. catharus* was rapid with pH restored to preimmersion levels within 4 hours and [lactate] returning to basal levels between 4 and 12 hours after reimmersion. The rapid recovery of haemolymph pH will be largely due to the restoration of the gas and ion exchange functions of the gills when returned to water. As mentioned earlier, lactate is removed from the haemolymph more slowly as it does not appear to be excreted (Phillips et al., 1977), but is metabolised back to glucose, or oxidised to CO_2 and H_2O.

Despite *O. catharus* showing a bigger reduction in $\dot{M}o_2$ in air than *J. edwardsii*, mortality was greater in the latter (Waldron, 1991). Four out of a group of 22 individuals of *J. edwardsii* died when emersed for 8 hours, giving an 18% mortality. In the present study, 1 of the 16 animals emersed for a 12 hour period died (Series I, 6% mortality).

The two animals that died during the Series II experiment could be identified at the first emersion sample time by their very high [lactate] values. These were roughly twice the mean value for the group (= 3.2 ± 0.6 mmol l^{-1}), at 6.3 and 5.2 mmol l^{-1}. Many of the surviving animals had final [lactate] values higher than these, the lowest recorded concentration at this time was 5.4 mmol l^{-1}. Thus, there does not appear to be a single threshold level of [lactate] above which death is certain, but rather individual tolerances may vary. Also the increased [lactate] seen before death may be secondary to some other causative factor. Animals which died did not show changes in haemolymph pH that were exceptional when compared with other crabs, and overall, after 6 hours in air, there was no significant correlation between pH and [lactate] (Spearman's rank correlation coefficient, $p > 0.05$).

The mean [lactate] from animals that were actually transported by air freight from Nelson to Christchurch was twice that recorded from animals emersed under controlled conditions for a similar period (6.4 ± 0.9 mmol l^{-1} after 5 hours and 3.2 ± 0.6 mmol l^{-1} after 6 hours, respectively). This presumably reflects the added stress of temperature fluctuations, desiccation and handling experienced by the animals in transit. Given this, it would seem to be reasonable to assume that any response observed under experimental conditions could be up to twice as great during an actual commercial operation. No effort was made to cool the animals prior to

transportation, and no doubt this would contribute to the comparatively high lactate levels recorded. Many commercial suppliers will briefly chill animals prior to packing. This treatment quietens the animals down and allows them to be handled more easily. At present, chilling is usually accomplished fairly crudely with animals being brought out into air, placed in a chiller, and then packed for shipment. As a result, the animals have struggled considerably before they become quiescent. Whether using chilling to anaesthetise the animals is beneficial remains unclear. Otwell and Webb (1997) recorded higher mortalities during emersion in the blue crab *Callinectes sapidus* at 5°C than at 10°C. Given an optimum temperature for *O. catharus*, chilling would ideally be performed in a manner similar to that used for *J. edwardsii*, with animals being cooled slowly over a number of hours while still immersed in sea water.

Heart and scaphognathite activity

Many previous studies investigating the condition of crustaceans undergoing commercial procedures, have relied upon visual examination of the outward appearance of the animals to assess their internal state (McLeese, 1985; Otwell and Webb, 1977; MacMullen et al., 1986). Such examination requires the animals to be handled, which will exacerbate disruptions of the internal acid-base balance (Waldron, 1991), and increase mortality (Otwell and Webb, 1977). Alternatively, visual inspection without disturbing the animals may be deceptive. Individuals of *O. catharus* appear quite moribund shortly following emersion, but they are instantly active if disturbed. Monitoring ventilatory and cardiac activity remotely by impedance techniques appears to provide an ideal means of monitoring an animals condition without confounding the effects of the experimental treatment. If the insertion of impedance electrodes has an effect (which is highly unlikely) then a truly non-invasive technique, such as photoplethysmography could be used instead (Depledge, 1984). This experiment also indicates the difficulty in defining the point of death in crustaceans. *O. catharus* is effectively dead when ventilation has ceased, as I have shown that animals will not recover if reimmersed at this stage. It is possible that the animals may be beyond recovery before this point is reached.

Waldron (1991) performed a similar experiment with *J. edwardsii*, where animals were emersed and forced to exercise. Each animal performed 50 tail flips before being left in air for a total period of 8 hours. The exercise period took between 1-4 minutes to complete. This would be comparable to the effects of handling upon emersion in *O. catharus* in the present study. In contrast to *O.*

catharus, *J. edwardsii* showed a reduction in both F_r and F_h following exercise in air. Animals in the present study experienced a large increase in temperature of between 5 and 7°C upon emersion, similar to that which may occur during packing for shipment. This is likely to have contributed to the observed rate increases. Taylor et al. (1973) demonstrated nearly a 3 fold increase in F_h, and a 2.5 fold increase in F_r, in immersed *Carcinus maenas*, with a temperature increase from 6°C to 17°C. Temperature was held constant in Waldrons study. Many decapods exhibit an increase in F_r during hypoxic exposure presumably in response to declining internal $pO2$ (McMahon and Wilkens, 1975; Butler et al., 1978; Burnett and Johansen, 1982; Bradford and Taylor, 1982; Waldron, 1991). deFur and McMahon (1984) argue that the increase seen in F_r in large *C. pagurus* during emersion occurs too rapidly to be a response to falling internal Po_2 and suggest that it may simply be a reflex response to emersion, similar to the bradycardia that occurs in terrestrial vertebrates upon immersion.

The response of F_r and F_h to emersion has been shown to be size dependant in a number of decapod species. For example, in the blue crab *C. sapidus*, large animals show an increase in F_r and F_h during emersion while small individuals (< 70 g) show a reduction in both variables (deFur et al., 1988). This size dependant response has also been observed in *Cancer pagurus* (deFur and McMahon, 1984) and the shore crab *Carcinus maenas* (deFur, 1982). The size range used in the present study was not large enough to identify such an effect. From a commercial aspect the consequences of size dependant emersion responses for survival would be relatively unimportant as only large crabs (> 250 g) are used for live transportation.

Overall, *O. catharus* seems to be able to cope with emersion as well as, if not better than *J. edwardsii*. Given that *J. edwardsii* is routinely transported live in air to overseas markets, it would appear that *O. catharus* should be able to undergo the same treatment with equal success.

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